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CONIDIAL FRUCTIFICATIONS IN BALANSIA AND DOTHICHLOE [1]

By WILLIAM W. DIEHL

Associate Pathologist, Office of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Balansia and Dothichloe are generally recognized as parasites upon grasses and sedges, although the conidial fructifications of these fungi have usually escaped observation because they are ephemeral and inconspicuous. These two genera have been distinguished chiefly by the form and position of the ascogenous fructification. The stroma of Balansia is defined as capitate and stipitate and that of Dothichloe as effuse. Dothichloe by definition has an effuse ascogenous stroma resembling Epichloe Tul. except that it is black. Certain species show intermediate characters in this respect, and there is little actual structural difference implied by these generic distinctions. Other structures such as conidial fructifications should be useful in clarifying the generic concepts, especially since so little has been known in regard to conidial fructifications in either genus. The importance of recognizing conidial stages is obvious, since their usually inconspicuous occurrence is overlooked even when correlated with floret sterility or abortion.

This paper presents certain observations on conidial phases thought to be significant in these genera. Cultural study of the organisms has been limited to moist-chamber development, since attempts at cultivation upon artificial media have thus far been ineffective. Ascospore germination followed by scattered conidial production, not in a fructification, as illustrated by Brefeld (*4, pl. 5, p. 59–62*) [2] for *Epichloe typhina* Tul. and by Moeller (*10, pl. 5*) for Balansia, are not discussed here because they are not regarded as especially pertinent. All specimens referred to have been deposited with the mycological collections of the Bureau of Plant Industry.

BALANSIA

BALANSIA CLAVICEPS AND EPHELIS MEXICANA

Balansia claviceps Speg. (*11, p. 45–46*), the type species of the genus, was described as having the capitate ascogenous stroma borne upon a sclerotium formed upon an unknown grass in Bolivia. No conidial fructifications were mentioned, nor has any conidial stage since been recorded for it.

Balansia trinitensis Cke. and Mass. (*5*) is a very similar species, described, however, with illustrations of a conidial fructification assigned to the form genus Ephelis as *E. trinitensis* Cke. and Mass. The conidia are said to be borne upon short, simple conidiophores in a characteristic discoid fructification.

[1] Received for publication July 23, 1930; issued December, 1930.

[2] Reference is made by number (italic) to Literature Cited, p. 766.

The form genus Ephelis Fr. (*9, p. 370*) is based upon *E. mexicana* Fr. sec. B. and C. (*3, p. 353*). This species grows upon various grassses in Mexico, Florida, and the West Indies. (Pl. 1.) Living material of *E. mexicana* growing on Cenchrus in Florida has been available for study at different times for several years through the kindness of G. F. Weber, A. J. Bruman, A. C. Foster, H. R. Fulton, and G. B. Sartoris. The Ephelis fructification develops from certain points beneath the black, laccate rind of the sclerotium as a globular, hairy protrusion which opens, becoming discoid. (Pl. 1, H–K.) The rim and lower surface of the disk are an exciple covered with long hairs (fig. 1)[3] 3μ to 4μ in diameter. The upper surface of the disk is a grayish layer of needle-shaped conidia borne as a palisade upon conidiophores at first simple, then verticillately branched. (Fig. 2.) Under certain conditions the conidia extrude in conical to columnar masses. When mature the fructifications are usually but not always discoid, simulating apothecia of an ascomycete. This similarity induced Fries (*9*) to describe the fungus as a discomycete. This type of fructification is usually regarded as a

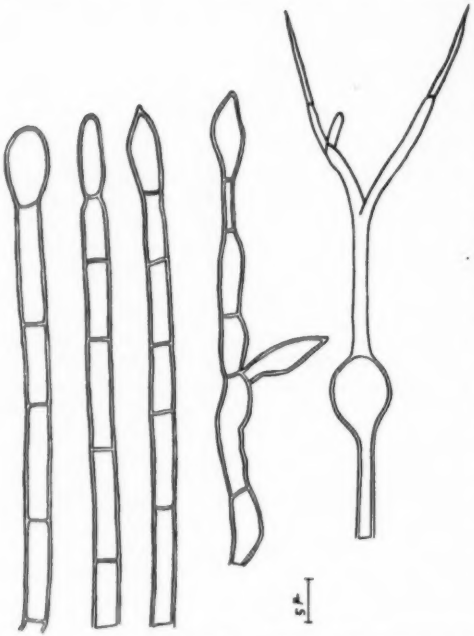
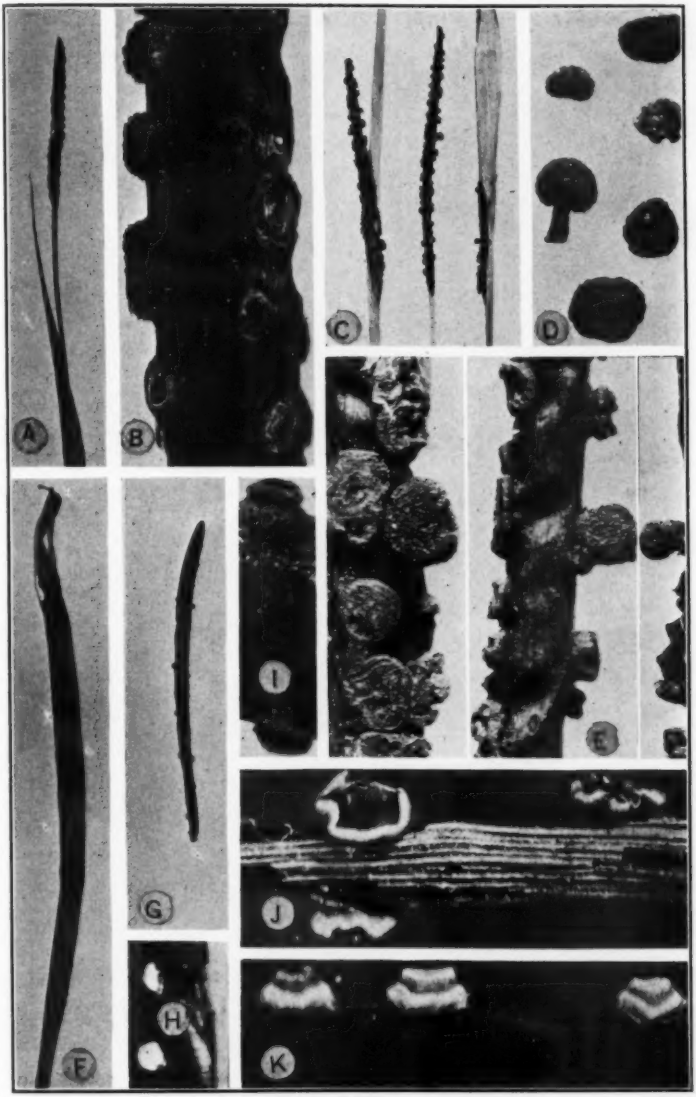


FIGURE 1.—Excipular hairs of *Ephelis mexicana*, aberrant branched hairs at the right, on Cenchrus from Florida

EXPLANATORY LEGEND FOR PLATE 1

A.—*Ephelis mexicana* on *Chaetochloa geniculata;* Central Hershey, Cuba, February 3, 1924, J. A. Stevenson; sclerotium covering the aborted inflorescence, and bearing typical fructifications. × 1.
B.—Detail of A. × 12.
C.—*Ephelis mexicana* on *Chaetochloa* sp.; Santiago de las Vegas, Cuba, August 29, 1907, H. Hasselbring; sclerotium bearing Ephelis and ascogenous (*Balansia* sp.) fructifications. × 1.
D.—Data of C.; detached ascogenous stromata. × 8.
E.—Detail of C. × 8.
F.—*Ephelis mexicana* on *Cenchrus echinatus;* Lake City, Fla., January 28, 1894, P. H. Rolfs; sclerotium covering the aborted inflorescence, partly inclosed by leaf sheath. × 1.
G.—*Ephelis mexicana* from *Cenchrus echinatus;* Gainesville, Fla., March 27, 1923, G. F. Weber; detached sclerotium bearing Ephelis fructifications. × 1.
H.—*Ephelis mexicana* on *Cenchrus echinatus;* Pierson, Fla., December 20, 1927; initial appearance of Ephelis fructifications on sclerotia developed in the moist chamber. × 12.
I.—Data of H; partially developed Ephelis fructifications after 24 hours in a moist chamber. × 12.
J.—Data of H; mature Ephelis fructifications. × 12.
K.—Data of I; mature Ephelis fructifications with adherent spore mass, spores here produced chiefly upon verticillately branched conidiophores. × 12.
Photographs for C, D, and E by J. F. Brewer; the others by M. L. S. Foubert.

[3] Drawings for figs. 1, 2, and 3 were made from living material mounted in water.



EPHELIS MEXICANA

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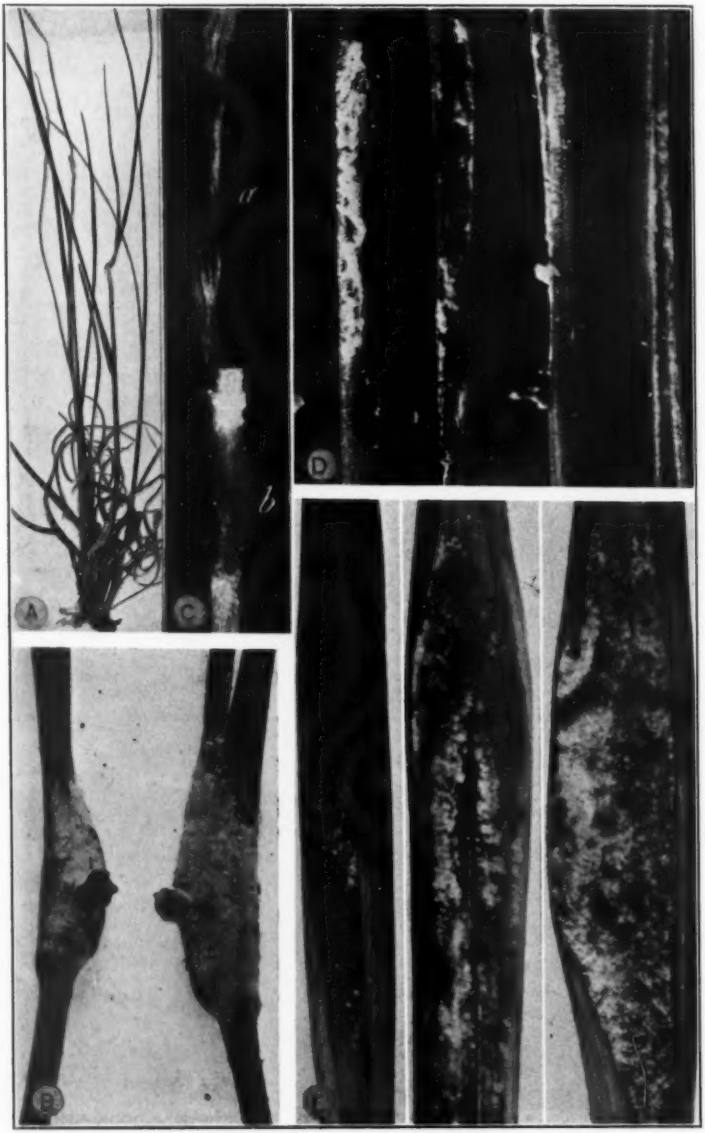

BALANSIA AND DOTHICHLOE

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pycnidium and assigned to the Excipulaceae, although its structure, disregarding the peculiar exciple, is suggestive of a sporodochium.

A noteworthy specimen of *Ephelis mexicana* on Chaetochloa gathered in Cuba in 1907 by H. Hasselbring is available through his kindness. It bears a few ascogenous stromata of Balansia. (Pl. 1, C and D.) This is most likely congeneric with a specimen of *Balansia claviceps* Speg. presented to the writer by the late Professor Spegazzini, but appears to be specifically distinct.

BALANSIA HYPOXYLON

Balansia hypoxylon (Pk.) Atk. (*2*) growing upon various grasses in the eastern half of the United States forms small whitish to grayish-green sclerotia surrounding the immature inflorescence, which it mummifies. (Pl. 2, A and B.)

The surface of the young sclerotium is made up of a palisade of swollen conidiophores, each with a single sterigmalike apical projection bearing an ovate conidium. (Fig. 3, A.) These conidia were observed by Atkinson (*2*) in a single specimen sent to him from Texas, but he was doubtful of their significance. This conidial layer and conidia are somewhat similar to and probably homologous with the Sphacelia stage which was shown by Tulasne for Epichloe (*12, p. 647*) and for Claviceps (*11, 13*). The conidiophores of Claviceps do not normally have apical projections or sterigmata as is the case in *Balansia hypoxylon*. In the closely related genus Epichloe there is, however, a sterigmalike apical projection on the swollen conidiophore, generally ignored in

FIGURE 2.—Conidia and conidiophores of *Ephelis mexicana* on Cenchrus from Florida

EXPLANATORY LEGEND FOR PLATE 2

A.—*Balansia hypoxylon* (diseased specimen of *Danthonia spicata*, Barcroft, Va., June 2, 1929, H. A. Allard), showing inflorescence covered by fungous tissue or sclerotium. × 1.

B.—*Balansia hypoxylon*, data of A, sclerotia upon aborted inflorescence covered by the grayish amerosporous fructification, each sclerotium bearing an Ephelis fructification. × 8.

C.—*Dothichloe aristidae* on *Panicum* sp.; Arlington Experiment Farm, Rosslyn, Va., June 20, 1929, W. W. Diehl: *a*, Effuse, grayish scolecosporous layer covering culm and aborted inflorescence (× 12); *b*, remnant of scolecosporous fructification with immature ascogenous stroma (× 9).

D.—*Dothichloe atramentosa* on *Sporobolus berteroanus* (Auburn, Ala., July 17, 1929, J. L. Seal), showing masses of scoleco spores extruding from between folds of the leaves. × 14.

E.*D—othichloe atramentosa*, data of D; amerosporous layer developed upon immature ascogenous stroma after exposure for 48 hours in a moist chamber. × 14.

Photographs for A and B by J. F. Brewer; others by M. L. S. Foubert.

illustrations but correctly shown in Delacroix's Atlas (*6, pl. 48, fig. 29*). These amerosporous fructifications of *B. hypoxylon* and of *Epichloe typhina* (*Sphacelia typhina* Sacc.) are practically identical, suggesting a close relationship. After the abscission of the conidia in *B. hypoxylon* the sclerotium remains gray to gray green in color, due to the covering of conidiophores. The sclerotium is at first gelatinous but becomes cartilaginous in texture; it never forms a laccate rind like that covering the sclerotium of *E. mexicana*.

These sclerotia later bear a discoid Ephelis stage (pl. 2, B), described originally from a Canadian specimen as *Ephelis borealis* E. and E. (*8, p. 86*). The fructification, scolecospores, and conidiophores are very similar to those of *E. mexicana*, but differ in that in the northern species the excipular hairs are interspersed with the peculiar ovate conidia. It appears that excipular hairs of *Balansia hypoxylon* may be homologous with elongated conidiophores which remain sterile, since hairlike sterile structures may be produced in mass upon the surface of the sclerotium when in contact with water. (Fig. 3, A.)

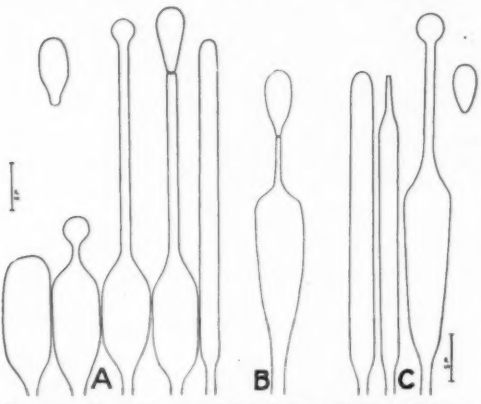

FIGURE 3.—Amerosporous conidia and conidiophores: A, *Balansia hypoxylon* (an aberrant conidiophore at the right) on Danthonia from Virginia; B, *Dothichloe atramentosa* on Sporobolus from Alabama; C, *D. aristidae* on Panicum from Virginia

These observations on *Balansia hypoxylon* are based upon living material growing upon *Danthonia spicata* obtained chiefly at Barcroft,[4] Arlington Co., Va., in 1929, as well as upon various dried herbarium specimens. At Barcroft both types of fructifications were first observed early in June. By July and August of 1929 the formation of ascogenous stromata had apparently completed the activities of the fungus for the season.

BALANSIA CYPERI

Balansia cyperi Edg. (*7*) has acicular, Ephelislike conidia in pink masses on indefinite areas of the surface of the white sclerotia. These conidial areas lack the definite excipular structure of Ephelis. The species has not been available to the present writer in a living condition, and hence it is not advantageous to discuss its relations further here.

DOTHICHLOE

The genus Dothichloe Atk. (*1, p. 223*), with *D. atramentosa* (B. and C.) Atk. (*2, p. 260–264*) as the type species, was distinguished because of its black, effuse ascogenous stroma. This species on grass blades appears to be common in the southeastern part of the United

[4] The writer is grateful to H. A. Allard for reporting this colony of diseased plants.

States and in the West Indies. In living material on *Sporobolus berteroanus* from Auburn, Ala., generously contributed in 1929 by J. L. Seal, it was observed that preceding the development of the ascogenous stroma and coincident with the unrolling of the young grass blade there is formed an effuse palisade layer of acicular conidia (scolecospores). From beneath the folds of the grass blade the conidia extrude in waxy masses. (Pl. 2, D.) Similar material in an early stage of development growing on an unknown grass at Burgaw, N. C., was observed by the writer on April 25, 1930. The conidia and conidiophores are practically identical with those of *Ephelis mexicana* and of the Ephelis stage of *Balansia hypoxylon*. This effuse conidial layer is, however, not circumscribed by any definite exciple. The examination of herbarium material of the species has disclosed the presence of the scolecosporous fructifications in specimens from various localities. This scolecospore stage is later displaced by the effuse ascogenous stroma. When the stroma, as it approaches maturity, is placed in a humid atmosphere for 12 to 48 hours it develops upon its surface a whitish to grayish-green amerosporous layer (pl. 2, D, and fig. 3, B) resembling that in *B. hypoxylon*.

A closely related species is *Dothichloe aristidae* Atk. (*1, p. 224*). It surrounds the culms of various grasses, appearing as a black equivalent of *Epichloe typhina*, and is broadly distributed in North and South America. Living material growing upon *Panicum* sp. at the Arlington Experiment Farm, near Rosslyn, Va., in 1929, showed a scolecosporous fructification (pl. 2, C) followed by the ascogenous stroma which under certain moisture conditions would produce an amerosporous stage. (Fig. 3, C.) The conidial fructifications of *D. aristidae* are therefore practically indistinguishable microscopically from those of *D. atramentosa* and develop in the same sequence.

SUMMARY

In *Balansia hypoxylon* and in the known species of Dothichloe, fungus parasites of certain grasses, conidial fructifications hitherto unknown or rarely recognized are found to develop in a similar manner.

An amerosporous conidial fructification similar to that of Epichloe is reported for two species of Dothichloe and for *Balansia hypoxylon*.

An ascogenous fructification referable to Balansia is reported for *Ephelis mexicana*, for which no amerosporous stage has been found.

An effuse conidial fructification microscopically similar to the Ephelis (scolecosporous) stage of Balansia is reported for Dothichloe.

Branched conidiophores are reported in the Ephelis fructifications of *Balansia hypoxylon*, in *Ephelis mexicana*, and in the scolecosporous fructifications of Dothichloe.

The character and sequence of the conidial fructifications in Balansia and in Dothichloe show definite correlations which suggest their recognition in the taxonomic interpretation of these fungi.

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FURTHER STUDIES ON THE RELATIONSHIP BETWEEN THE CONCENTRATION OF THE SOIL SOLUTION AND THE PHYSICOCHEMICAL PROPERTIES OF THE LEAF-TISSUE FLUIDS OF COTTON [1]

By J. ARTHUR HARRIS,[2] *Head, Department of Botany, University of Minnesota, and Collaborator, Office of Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture,* and TRUMAN A. PASCOE, formerly *Field Assistant, Office of Egyptian Cotton Breeding, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

While the problem of the relationship between the chemical and physical properties of the soil and the characteristics of the plant is as old as the beginnings of scientific agriculture, it is only recently that the investigation of certain phases of the problem by means of the determination of correlations between the properties of the soil and the characteristics of crop plants as grown in the experimental field has been undertaken.

In the many studies that have been made of the influence of the nutrient solution on the characteristics of the plant, the method almost universally employed has been that of culture in synthetic solutions of known composition. Unquestionably this technic has yielded results of great importance. It is also quite clear that this method must furnish the final criterion for the determination of the rôle of various elements in the metabolism of the plant.

There are, however, many difficulties connected with investigations involving such methods. Plants are grown under conditions very different from those that are normal for their development. Notwithstanding the many efforts that have been made to eliminate the influence of these abnormalities of condition on the development of the organism, it is quite clear that plants grown in culture solutions do not develop in exactly the same way as those grown in the field. It therefore seems desirable to supplement water-culture and pot-culture methods by others whenever possible. One such accessory method is to consider the relationship between soil properties in the field and the characteristics of the plants produced. While field experiments can not be expected to give results of the same order of finality as water-culture and pot-culture methods in the determination of the relative importance of individual constituents of the soil solution in the metabolism of the plant, they nevertheless have two advantages:

(1) The investigations are conducted on plants growing under natural conditions. Any results secured will, therefore, be such as may be expected in the actual growth of crop plants in the field.

(2) By the proper development of agronomic, chemical, and biometric technic, the investigations may be carried out simultaneously

[1] Received for publication June 13, 1930; issued December, 1930. The term "soil solution" is preferred to "soil extract" in this paper since the argument throughout is directed toward the content of chlorides and sulphates which are present in the soil as the environment of root growth.

[2] Died Apr. 24, 1930.

with others on crop plants as grown under conditions of agriculture, or at least of large-scale field experimentation. This combination of agronomic and physiological technic has obvious practical advantages.

The purpose of the present paper is to report further investigations on the relationship between the concentration of the soil solution and the tissue-fluid properties of the Egyptian and upland types of cotton.

The first study (*6*)[3] dealt with the relationship between the electrical resistance of the soil on the one hand and the osmotic concentration (as expressed in terms of freezing-point depression), specific electrical conductivity, and chloride content of the leaf-tissue fluids of the plant on the other.

In a subsequent paper (*8*) evidence was adduced to show that under certain conditions there is a positive correlation between soil salinity and seedling stand in cotton. Finally it seemed desirable to extend investigations to the consideration of the relationship between the properties of the soil and some functional characteristics of the plant. An analysis (*9*) of records of Pima Egyptian, Meade upland, and Acala upland cotton indicates that the flowering date is slightly retarded by soil salinity.

In the earlier investigation (*6*) the electrical resistance of the saturated soil mass, as determined by the electrical bridge in the form described by Davis and Bryan (*4*), provided the only available measure of the concentration of the soil solution. Although this method was not regarded as altogether satisfactory, it seemed desirable to employ it in a preliminary investigation, rather than the much more expensive analytical methods. While the soil bridge has been extensively used in soil surveys and in other investigations, it is quite evident from the theory involved that it can not give information concerning the identities of the solutes to which the conductance of the saturated soil mass is due. This limitation has always been recognized. It is, therefore, desirable that actual analytical determinations be made of the solutes of the soil to which its electrical conductivity is due. In this paper the studies of soil salinity are extended to include analytically determined chloride and sulphate contents.

The determination of these ions is particularly desirable in any consideration of the relationship between the salinity of the soil and the characteristics of cotton. Not only is the cotton plant capable of high salt tolerance, as indicated by general agricultural experience, as suggested by observations made by Balls (*2*, *3*), and as established in early studies by Kearney and Means (*15*) and by Kearney (*14*), but the salt relations of the tissue fluids have been shown to be of peculiar interest. Investigations made for the Bureau of Plant Industry have shown not only that the chloride content (*12*) and the sulphate content (*10*) of the leaf-tissue fluids are high, but also that there is differential absorption of these anions (*5*, *7*, *11*, *13*). Furthermore, the chloride content of the tissue fluids, especially in the Egyptian type of cotton (*6*), has already been shown to be highly correlated with the electrical resistance of the soil.

[3] Reference is made by number (italic) to Literature Cited, p. 788.

For these reasons it seemed particularly desirable that an investigation be made of the relationship between the concentration of the chloride and sulphate ions in the soil on the one hand and the physicochemical properties, especially the chloride and sulphate contents of the leaf-tissue fluids, on the other.

METHODS

DETERMINATION OF SOIL CONSTITUENTS

The electrical resistance of the saturated soil mass was measured by the conventional method as indicated above.

The chloride and the sulphate content were determined on 30 to 50 gm. fractions of the air-dried, finely pulverized, and thoroughly mixed soil. These were transferred to 300 c. c. Florence flasks and 200 c. c. of a 5 per cent solution of ammonium nitrate (NH_4NO_3) was added to each flask. After 24 hours, during which time the suspensions were agitated frequently to produce a uniform suspension, a clear soil extract was obtained by filtering through quantitative filter paper. Through the use of the 5 per cent NH_4NO_3 solution as a solvent, the soil colloids were precipitated, and little difficulty was encountered in the subsequent filtrations. In the determination of chloride content, duplicate 25 c. c. portions of the filtrate were titrated against a tenth-normal solution of silver nitrate ($Ag\ NO_3$), with 1 c. c. of a 5 per cent solution of potassium chromate (K_2CrO_4) as an end-point indicator (*1*, *p. 87*). In the determination of sulphate content, 100 c. c. portions of the filtered soil extract (obtained as above) were acidified with 5 c. c. concentrated hydrochloric acid (HCl) and heated to boiling on a hot plate. To the hot solution 10 c. c. of a 10 per cent solution of barium chloride ($BaCl_2$) was added with constant stirring, and the boiling was continued until the precipitated barium sulphate ($BaSO_4$) settled to the bottom of the beaker. The precipitate was collected on a quantitative filter paper, washed, dried, and ashed to constant weight in platinum.

DETERMINATION OF TISSUE-FLUID PROPERTIES

The methods employed are those which have been used in the various investigations on the tissue-fluid properties of cotton discussed in papers already published (*5*, *6*, *10*, *12*, *13*).

STATISTICAL ANALYSIS OF DATA

Correlation between soil properties and tissue-fluid properties have been determined by the usual product-moment method. Special methods of comparison will be indicated as required.

MATERIALS

The cultures considered are those of an experiment made in 1923. This set of cultures has furnished data on the chloride content (*12*) and the sulphate content (*10*) of the tissue fluids and on the relationship between soil salinity and seedling stand (*8*). The last-named paper (*8*) may be consulted for details of arrangement of plantings. In the consideration of the relationship between soil salinity and seedling stand (*8*) the area of the field used was limited to 16 rows, of 90 hills each, of the two varieties of cotton studied. (Hybrid

plants grown between the sections or plots of the two parental types were disregarded.) This course was followed because conditions at the north half of the plots were such that only a very few hills produced seedlings at all. To have included this area would have resulted in highly abnormal distributions of the number of seedlings per hill, since high modal frequencies on the zero class would have been obtained.

In the present investigation it has seemed desirable to include the determinations made on the few plants growing on the extremely saline north half of the experimental plots (D 1–10, D 1–11). This will be more readily understood if constants for the tissue fluids are first explained.

As noted in the papers on chloride content (*12*) and sulphate content (*10*), two series of determinations were made on the leaf-tissue fluids, the first based on collections taken from July 29 to August 14, and the second on samples taken from August 18 to 31.

The first series of determinations was made only on the plants on the south half of the plots, since those on the north half were so small that it was quite evident that samples of tissues for two sets of analyses would not be available. In consequence of this limitation, the correlations of the present paper are based in part on plants grown on the south half of the plot and in part on plants grown on both halves of the plot. Series 1 includes the correlations based on the first set of determinations of tissue-fluid properties. Series 2–B includes the correlations based on the second set of determinations but limited to the south half of the plot. It is therefore exactly comparable with series 1 as far as experimental area is concerned. Series 2–A includes all the tissue-fluid determinations based on plants growing on both the north and the south halves of the experimental plot.

RESULTS

All the correlation coefficients expressing the relationship between the measures of soil salinity and the properties of the leaf-tissue fluids are presented in Tables 1 to 3. The first column gives the variables of the soil (electrical resistance, R; chloride content, Cl; and sulphate content, SO_4) and of the plant (freezing-point depression, Δ; specific electrical conductivity, κ; chloride content, Cl; and sulphate content, SO_4) involved in the correlations denoted by the symbols $r_{R\Delta}$, $r_{R\kappa}$, r_{RCl}, r_{RSO_4}, etc. The third to the tenth columns, inclusive, give the coefficients based on the determinations made on the soil layers of the first 4 feet. The eleventh and twelfth columns give the coefficients based on the average resistance of the first 4 feet. Finally, the thirteenth and fourteenth columns give the average of the coefficients based on the constants for the four individual soil layers.

As indicated above, two sets of determinations on the leaf-tissue fluids were available. Table 1 gives the coefficients measuring the relationship between the properties of the soil and the first series of determinations on the leaf-tissue fluids. Tables 2 and 3 give comparable results for the second series of determinations. Table 3 presents the constants for the south half of the field. It is therefore altogether comparable with Table 1. Table 2 gives the data from the second series of determinations for both parts of the experimental plot. These coefficients represent the fundamental constants upon which the subsequent discussion is based.

TABLE 1.—*Correlation coefficients measuring the relationship between each of the three measures of soil salinity (electrical resistance, chloride content, and sulphate content) and various tissue-fluid properties in Pima Egyptian and Lone Star upland cotton grown at the United States Field Station, Sacaton, Ariz., in 1923*

[Experiment 1/23; series 1, determinations based on plants grown on south half of plot]

Variables	Correlation	Coefficient for—										Average correlation coefficient	
		First foot [a]		Second foot [a]		Third foot [a]		Fourth foot [a]		All layers [b]			
		Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland
Resistance and—													
Δ	$r\ R\ \Delta$	−0.3214± .0762	−0.1772± .0830	−0.4611± .0669	−0.2221± .0814	−0.4540± .0675	−0.3116± .0773	−0.4520± .0676	−0.2925± .0783	−0.5044± .0634	−0.3008± .0779	−0.4221	−0.2508
κ	$r\ R\kappa$	−.2228± .0821	−.1741± .0831	−.5350± .0616	−.1014± .0848	−.4091± .0719	−.0429± .0855	−.3641± .0749	−.0329± .0856	−.4677± .0675	−.0988± .0848	−.3828	−.0878
Cl	$r\ R\ Cl$	−.1203± .0838	−.0330± .0849	−.2789± .0784	+.0210± .0849	−.4559± .0673	−.1320± .0835	−.4624± .0668	−.0798± .0844	−.4094± .0707	−.0774± .0845	−.3294	−.0560
SO_4	$r\ R\ SO_4$	−.0967± .0842	−.2894± .0778	−.3197± .0763	−.2358± .0802	−.3226± .0761	−.2028± .0815	−.1296± .0836	−.2996± .0774	−.2547± .0795	−.3027± .0772	−.2172	−.2569
Chloride and—													
Δ	$r\ Cl\ \Delta$	+.2512± .0844	+.1862± .0878	+.2644± .0817	+.2468± .0832	+.3589± .0740	+.2636± .0797	+.3414± .0776	+.4344± .0718			+.3040	+.2828
κ	$r\ Cl\ \kappa$	+.1694± .0892	+.1047± .0900	+.3303± .0796	+.1023± .0876	+.3420± .0763	+.0953± .0849	+.2438± .0840	+.0856± .0879			+.2714	+.0970
Cl	$r\ Cl\ Cl$	+.1442± .0882	+.1163± .0881	+.1145± .0866	−.0430± .0876	+.2725± .0787	+.0576± .0847	+.3906± .0744	+.2020± .0842			+.2304	+.0832
SO_4	$r\ Cl\ SO_4$	+.2690± .0836	+.0916± .0894	+.2576± .0820	+.2382± .0828	+.2306± .0805	+.0878± .0843	+.1186± .0866	+.1240± .0865			+.2190	+.1354
Sulphate and—													
Δ	$r\ SO_4\Delta$	+.2848± .0828	+.2540± .0851	+.5108± .0649	+.0707± .0881	+.4812± .0653	+.0941± .0849	+.4620± .0691	+.1114± .0875			+.4347	+.1326
κ	$r\ SO_4\kappa$	+.1870± .0886	+.0362± .0908	+.5723± .0601	+.0430± .0884	+.3824± .0737	−.0044± .0856	+.2342± .0830	−.1092± .0875			+.3440	−.0086
Cl	$r\ SO_4Cl$	+.2455± .0847	−.0069± .0901	+.2131± .0838	−.1353± .0862	+.2078± .0813	−.2053± .0814	+.3315± .0782	−.1707± .0852			+.2495	−.1295
SO_4	$r\ SO_4SO_4$	+.2160± .0859	+.1100± .0890	+.2932± .0803	+.1704± .0853	+.2283± .0805	+.1291± .0836	+.1635± .0855	+.1482± .0859			+.2253	+.1394

[a] Depth of soil sample.

[b] Calculated from the average values of the individual determinations of the first to fourth foot samples, inclusive.

TABLE 2.—*Correlation coefficients measuring the relationship between each of the three measures of soil salinity (electrical resistance, chloride content, and sulphate content) and various tissue-fluid properties in Pima Egyptian and Lone Star upland cotton grown at the United States Field Station, Sacaton, Ariz., in 1923*

[Experiment 1/23; series 2-A, determinations based on plants grown on the entire plot]

Variables	Correlation	Coefficient for—										Average correlation coefficient	
		First foot [a]		Second foot [a]		Third foot [a]		Fourth foot [a]		All layers [b]			
		Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland
Resistance and—													
Δ	$rR\ \Delta$	−0.4126± .0670	−0.4608± .0664	−0.5194± .0616	−0.4338± .0684	−0.4374± .0682	−0.4697± .0657	−0.4719± .0655	−0.4461± .0675	−0.5240± .0612	−0.5205± .0615	−0.4603	−0.4526
κ	$rR\ \kappa$	−.4743± .0653	−.3478± .0741	−.4500± .0672	−.3228± .0755	−.4823± .0647	−.3544± .0737	−.5062± .0627	−.3046± .0765	−.5597± .0579	−.3912± .0714	−.4782	−.3324
Cl	$rR\ Cl$	−.1483± .0824	−.2706± .0781	−.3063± .0764	−.2247± .0800	−.4849± .0645	−.2638± .0784	−.4986± .0633	−.2132± .0805	−.4314± .0686	−.2773± .0778	−.3596	−.2431
SO_4	$rR\ SO_4$	−.1935± .0812	−.3445± .0743	−.3140± .0760	−.3611± .0733	−.2024± .0808	−.3292± .0752	−.3449± .0743	−.3862± .0717	−.2988± .0768	−.3948± .0712	−.2637	−.3552
Chloride and—													
Δ	$rCl\ \Delta$	+.4491± .0733	+.4323± .0746	+.5745± .0583	+.5283± .0628	+.6579± .0482	+.7354± .0390	+.7313± .0408	+.8134± .0297			+.6032	+.6274
κ	$rCl\ \kappa$	+.3547± .0802	+.2473± .0862	+.4791± .0671	+.4978± .0655	+.4838± .0651	+.5417± .0600	+.6152± .0546	+.6458± .0512			+.4832	+.4831
Cl	$rCl\ Cl$	+.2737± .0849	+.3125± .0828	+.2025± .0835	+.0969± .0863	+.2802± .0783	+.4262± .0695	+.3828± .0749	+.4010± .0737			+.2848	+.3092
SO_4	$rCl\ SO_4$	+.1632± .0893	+.2619± .0855	+.3430+ .0768	+.4082± .0726	+.3171± .0764	+.3203± .0763	+.4979± .0660	+.4610± .0691			+.3303	+.3628
Sulphate and—													
Δ	$rSO_4\Delta$	+.1076± .0868	+.3946± .0775	+.5138± .0641	+.4353± .0706	+.3793± .0728	+.3283± .0758	+.3950± .0741	+.2898± .0804			+.3489	+.3620
κ	$rSO_4\kappa$	+.2737± .0849	+.1768± .0889	+.3880± .0740	+.3214± .0781	+.3729± .0732	+.1684± .0826	+.2341± .0830	+.0930± .0870			+.3172	+.1899
Cl	rSO_4Cl	+.1179± .0905	+.3738± .0790	+.2023± .0835	+.2427± .0820	+.2662+ .0790	+.1129± .0839	+.2569± .0820	+.2133± .0838			+.2108	+.2357
SO_4	rSO_4SO_4	+.2559± .0858	+.2415± .0864	+.3511± .0763	+.3045± .0790	+.1790± .0822	+.1198± .0838	+.2007± .0843	+.2613± .0818			+.2467	+.2318

[a] Depth of soil sample.

[b] Calculated from the average values of the individual determinations of the first to fourth foot samples, inclusive.

TABLE 3.—*Correlation coefficients measuring the relationship between each of the three measures of soil salinity (electrical resistance, chloride content, and sulphate content) and various tissue-fluid properties in Pima Egyptian and Lone Star upland cotton grown at the United States Field Station, Sacaton, Ariz., in 1923*

[Experiment 1/23; series 2-B, determinations based on plants grown on south half of plot]

Variables	Correlation	Coefficient for—										Average correlation coefficient	
		First foot [a]		Second foot [a]		Third foot [a]		Fourth foot [a]		All layers [b]			
		Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland	Egyptian	Upland
Resistance and—													
Δ	$rR\,\Delta$	−0.1492± .0858	−0.2346± .0830	−0.3686± .0759	−0.1843± .0848	−0.1711± .0852	−0.2175± .0836	−0.2448± .0825	−0.1734± .0852	−0.2669± .0816	−0.2424± .0826	−0.2334	−0.2025
κ	$rR\,\kappa$	−.0034± .0878	−.1038± .0869	−.0031± .0878	−.0211± .0878	−.0032± .0878	−.0422± .0876	−.0036± .0878	−.0567± .0875	−.0041± .0878	−.0443± .0876	−.0034	−.0560
Cl	$rR\,Cl$	−.0539± .0876	−.0482± .0876	−.2523± .0822	−.0009± .0878	−.4567± .0695	−.0564± .0875	−.4683± .0686	+.0091± .0878	−.3877± .0746	−.0312± .0877	−.3078	−.0241
SO_4	$rR\,SO_4$	+.0607± .0875	−.1988± .0843	−.1312± .0863	−.2374± .0829	+.0423± .0876	−.1723± .0852	−.1654± .0854	−.2471± .0824	−.0498± .0876	−.2321± .0831	−.0484	−.2139
Chloride and—													
Δ	$rCl\,\Delta$	+.2368± .0900	+.1433± .0934	+.3642± .0789	+.2163± .0867	+.2470± .0824	+.2848± .0807	+.2151± .0875	+.3202± .0824			+.2658	+.2411
κ	$rCl\,\kappa$	+.2127± .0911	+.0023± .0954	+.1788± .0880	+.0485± .0907	+.1573± .0856	−.0535± .0876	+.1639± .0893	−.0648± .0914			+.1782	−.0169
Cl	$rCl\,Cl$	+.2337± .0902	+.0003± .0954	+.0912± .0902	−.1341± .0893	+.3278± .0784	+.0139± .0878	+.3720± .0791	−.0023± .0918			+.2562	−.0305
SO_4	$rCl\,SO_4$	−.1885± .0920	+.0465± .0952	+.1809± .0880	+.2801± .0838	+.0022± .0878	+.1768± .0851	+.1570± .0895	+.1497± .0897			+.0379	+.1633
Sulphate and—													
Δ	$rSO_4\,\Delta$	+.0774± .0948	+.2660± .0886	+.2790± .0839	+.0710± .0905	+.1646± .0854	+.0055± .0878	+.3532± .0803	+.1312± .0902			+.2185	+.1184
κ	$rSO_4\,\kappa$	+.3885± .0810	+.0333± .0953	+.1299± .0894	−.1511± .0889	+.2232± .0834	−.2033± .0842	+.1542± .0896	−.1418± .0899			+.2239	−.1157
Cl	$rSO_4\,Cl$	+.1534± .0931	−.0290± .0953	+.1239± .0896	−.0012± .0910	+.2337± .0830	−.1100± .0867	+.2617± .0855	+.0240± .0917			+.1932	−.0290
SO_4	$rSO_4\,SO_4$	+.0222± .0953	+.1507± .0932	+.1728± .0882	+.1198± .0896	+.0258± .0878	−.0030± .0878	+.0899± .0910	+.1972± .0882			+.0777	+.1162

[a] Depth of soil sample.

[b] Calculated from the average values of the individual determinations of the first to fourth foot samples, inclusive.

The data may be most readily understood if presented graphically as in Figure 1 for the first series and in Figures 2 and 3 for the second series of determinations. Figure 3 contains determinations for only the south half of the plot and is therefore quite comparable with Figure 1. Figure 2 represents correlations based on the total material from the less saline south half of the experimental area and on the few

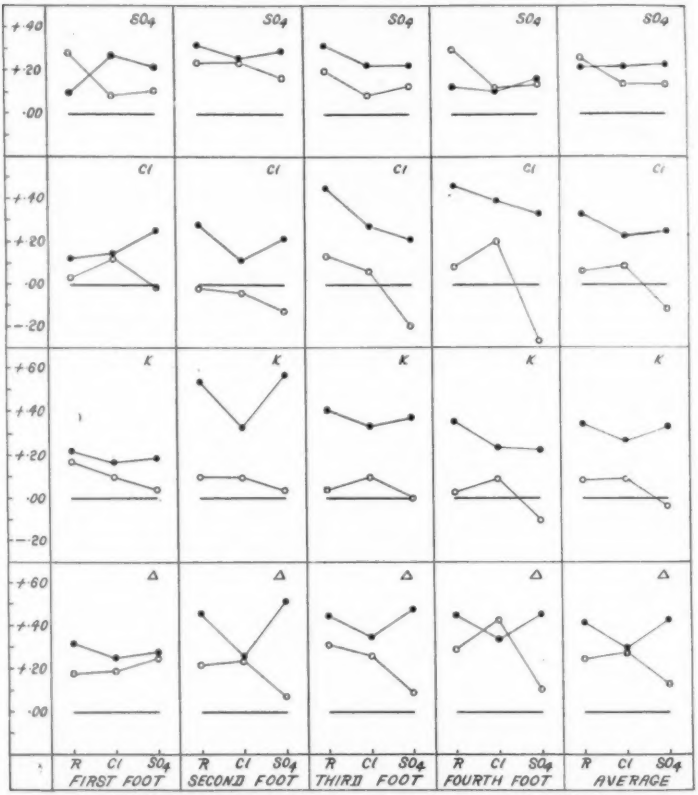

FIGURE 1.—Correlation coefficients measuring the relationship between each of the three measures of soil salinity and various tissue-fluid properties in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23). First series of determinations, based on south half of plot

available determinations from the highly saline north half of the experimental tract.

In these figures the magnitudes of the correlation coefficients are represented on the scale of ordinates. With the exceptions the correlations between soil resistance and the tissue-fluid properties, as given in the table of constants, have negative signs. Since soil salinity is measured in terms of soil resistance and is therefore, within limits, inversely proportional to the resistance, a negative coefficient indicates that higher soil salinities are associated with higher values of osmotic

concentration and electrical conductivity and higher chloride and sulphate contents of the tissue fluids. In representing these correlations in connection with the others the sign has therefore been changed in order to facilitate comparisons between the several different relationships considered and to reduce the vertical dimensions of the diagrams to a size practicable for publication.

In Figures 1 to 3 the soil property involved is indicated on the axis of abscissas, at the bottom of the vertical sections of panels, as *R*,

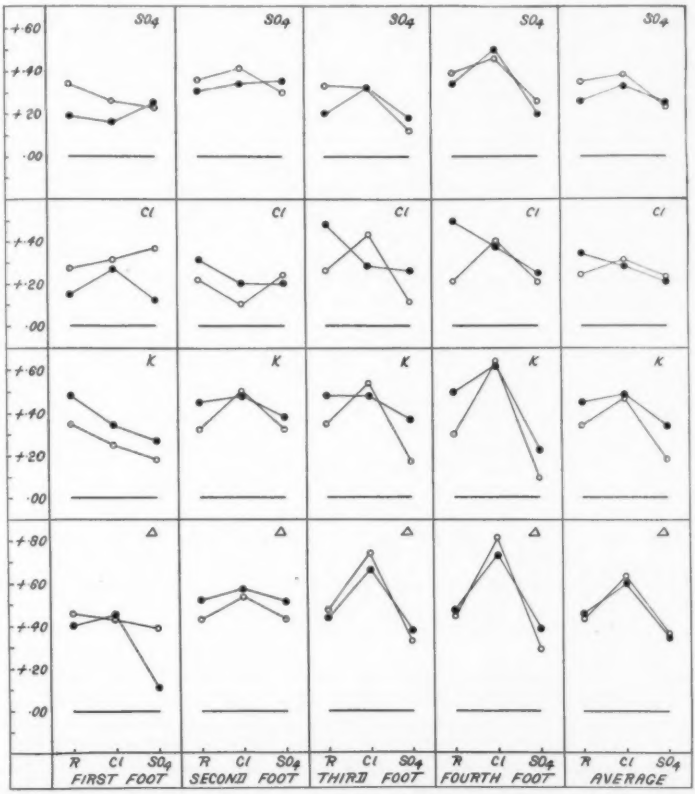

FIGURE 2.—Correlation coefficients measuring the relationship between each of the three measures of soil salinity and various tissue-fluid properties in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23). Second series of determinations, based on entire plot

Cl, and SO_4 for the first, second, third, and fourth foot. The right-hand sections of the figures represent the average magnitudes of the correlation coefficients (not the correlation coefficients for the averages of the soil properties) for the four different soil layers and the plant characteristics.

Figures 1 to 3 are divided into four transverse sections, each showing the relationships between a given property of the soil (*R*, *Cl*, or SO_4) and the osmotic concentration as measured in terms of freezing-

point depression (Δ), specific electrical conductivity (κ), chloride content (Cl), and sulphate content (SO_4) of the tissue fluids. The correlation coefficients for Pima Egyptian cotton are represented by the position of solid dots, while those for Lone Star upland cotton are shown by the position of circles, on the scale of ordinates. The position of these dots and circles with reference to the transverse bars that

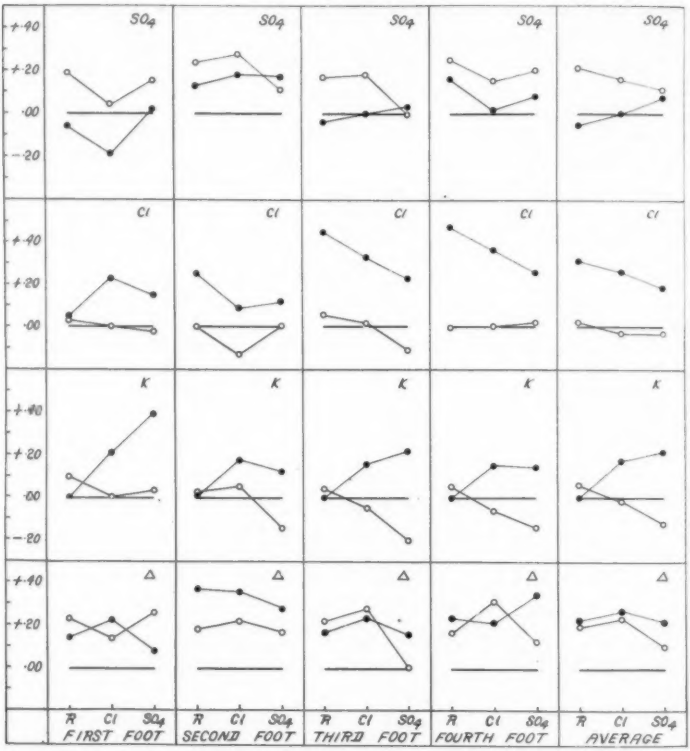

FIGURE 3.—Correlation coefficients measuring the relationship between each of the three measures of soil salinity and various tissue-fluid properties in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23). Second series of determinations, based on south half of plot

represent zero correlation will make the signs and the magnitudes of the coefficients quite clear.

Considering first the relationship between each of the three measures of soil salinity and the freezing-point depression (Δ) of the leaf-tissue fluids, as shown in the lowest transverse section of Figures 1 to 3, it appears that while the coefficients are distributed with some irregularity, all are positive values. It is interesting to note that in 25 of the 36 constants, exclusive of averages, the coefficient for Pima Egyptian cotton is higher than that for Lone Star upland cotton, whereas in 11 of the cases the reverse is true. This point will be considered later.

Inspection of the transverse section, second from the bottom (figs. 1 to 3), representing the relationship between the properties of the soil and the specific electrical conductivity (κ) of the tissue fluids, shows that, in general, there is a positive correlation between the measures of the electrolyte content of the soil and the associated electrolyte content of the plant-tissue fluids.

In the first series of determinations (fig. 1) the coefficients for Pima Egyptian cotton are conspicuously higher than those for Lone Star upland cotton. As a matter of fact, the latter deviate only slightly from zero correlations. Their general average is actually negative in sign in the case of the relationship between the sulphate content of the soil and the electrical conductivity of the tissue fluids. If the second series of determinations is limited to the same area as the first (fig. 3), essentially the same results will be found, although it is to be noted that the correlations for soil resistance and the specific electrical conductivity of the tissue fluids of Pima Egyptian cotton are sensibly 0 for all levels, while the correlations for Lone Star upland cotton are only slightly positive. If the entire area of the experimental plot is included (fig. 2) it appears that all the coefficients are conspicuously positive and of the general order of magnitude $r = 0.40$. These relationships will be considered in greater detail after the correlations for the two individual electrolytes of the tissue fluids are discussed.

The relationships between soil salinity and the chloride content (*Cl*) of the leaf-tissue fluids are of especial interest since the Egyptian and the upland cottons have been shown to be differentiated with respect to chloride content (*7*, *11*, *12*, *13*) and sulphate content (*7*, *10*, *13*). In the 36 determinations of both the first and second series, the correlations between the three measures of the concentration of the soil solution and the chloride content of the leaf-tissue fluids based on the Egyptian plants are positive. In the first series of determinations (fig. 1) and in those determinations of the second series that are based on the less saline area of the field (fig. 3), the correlations for the Egyptian cotton are higher in all cases than those for the upland variety. This is not uniformly the case if the correlations include those determinations of the second series that are based on the more saline area of the field (fig. 2).

While the correlations between the three measures of soil salinity and the chloride content of the Egyptian variety are positive in all cases, this condition is not found to prevail for the upland variety, for which a number of the coefficients are essentially 0 or even negative in sign. While great weight should not be put on these results, they nevertheless furnish a strong suggestion that the Egyptian type not only absorbs larger quantities of chlorides (as shown in previous investigations) than does the upland type, but that it is more exactly adjusted to the chloride content of the substratum than is the upland type of cotton. It may be urged in criticism of this view that only one-third of these coefficients measure the relationship between the chloride content of the soil and the chloride content of the leaf-tissue fluids, while the others measure the relationship between the electrical resistance and sulphate content of the soil on the one hand and the chloride content of the leaf-tissue fluids on the other. In reply to this suggestion, it must be pointed

out that the electrical resistance of the soil is in part inversely proportional to its chloride content and that there is in all probability a correlation between the chloride content and the sulphate content of the individual areas of the field. Thus a full solution of the problem under consideration involves a study of the correlations between each soil property and all the other soil properties as well as of those between the soil properties on the one hand and tissue-fluid properties on the other. It has not yet been possible to undertake this special investigation.

Finally, the relationship between the three measures of soil salinity and the sulphate content (SO_4) of the leaf-tissue fluids must be considered.

Figure 1 shows that for the first series of determinations the coefficients are positive in all cases. For the second series of determinations based on the same area (fig. 3) this is also generally true. In the latter case, however, it is to be noted that a number of the determinations for Egyptian cotton fall near or even below the line denoting zero correlation. When the correlations include determinations based on plants from the more saline portion of the field (fig. 2) all the coefficients are positive and of the order of magnitude $r = 0.10$ to 0.50.

In comparing the magnitudes of the correlations for sulphate content in the Egyptian and upland types, it is to be noted that in only 16 out of the 36 cases is the correlation for the Egyptian variety higher than that for the upland variety. In 20 of the cases the reverse is true.[4]

While the differences between the correlations for the sulphate content of the Egyptian and upland type are not large, it is to be noted that they are the reverse of those found for the correlations for the chloride content of the tissue fluids. The results suggest that the correlation between the soil salinity and sulphate content may be higher in the upland than in the Egyptian type. This must, however, remain merely a suggestion until further data are available.

Taking all the foregoing results as a whole, they show clearly that it is quite possible to deal in a quantitative way with the relationships between the analytically determined values of the ions of the soil and the characteristics of the plants when the latter are grown under ordinary field conditions. While the correlations are low in magnitude and somewhat irregular in distribution, this is unquestionably in part due to the relatively rough methods employed in all stages of this preliminary investigation. When methods of sampling and of the determination of the soil properties are improved, data of a much higher degree of precision may be secured.

The preceding discussion has been limited to a consideration of the general order of magnitude of the coefficients measuring the relationships between the properties of the soil and the physicochemical properties of the leaf-tissue fluids in the two varieties. It is now necessary to consider the relative magnitudes of the several classes of correlations. Two groups of comparisons are necessary for a full understanding of the results.

[4] The differences in the coefficients are so slight in some cases that they can not be read from the diagrams. They have, therefore, been summarized from Tables 1–3. In the correlations for soil resistance the negative signs have been made positive for purposes of comparison.

(1) The differences

$^{r}R\Delta - {}^{r}R\kappa$	$^{r}Cl\Delta - {}^{r}Cl\kappa$	$^{r}SO_4\Delta - {}^{r}SO_4\kappa$
$^{r}R\Delta - {}^{r}RCl$	$^{r}Cl\Delta - {}^{r}ClCl$	$^{r}SO_4\Delta - {}^{r}SO_4Cl$
$^{r}R\Delta - {}^{r}RSO_4$	$^{r}Cl\Delta - {}^{r}ClSO_4$	$^{r}SO_4\Delta - {}^{r}SO_4SO_4$
$^{r}R\kappa - {}^{r}RCl$	$^{r}Cl\kappa - {}^{r}ClCl$	$^{r}SO_4\kappa - {}^{r}SO_4Cl$
$^{r}R\kappa - {}^{r}RSO_4$	$^{r}Cl\kappa - {}^{r}ClSO_4$	$^{r}SO_4\kappa - {}^{r}SO_4SO_4$
$^{r}RCl - {}^{r}RSO_4$	$^{r}ClCl - {}^{r}ClSO_4$	$^{r}SO_4Cl - {}^{r}SO_4SO_4$

show the relative magnitudes of the correlations between each of the tissue-fluid properties and each of the three measures of the concentration of the soil solution.

(2) The differences

$^{r}R\Delta - {}^{r}Cl\Delta$	$^{r}R\kappa - {}^{r}Cl\kappa$	$^{r}RCl - {}^{r}ClCl$	$^{r}RSO_4 - {}^{r}ClSO_4$
$^{r}R\Delta - {}^{r}SO_4\Delta$	$^{r}R\kappa - {}^{r}SO_4\kappa$	$^{r}RCl - {}^{r}SO_4Cl$	$^{r}RSO_4 - {}^{r}SO_4SO_4$
$^{r}Cl\Delta - {}^{r}SO_4\Delta$	$^{r}Cl\kappa - {}^{r}SO_4\kappa$	$^{r}ClCl - {}^{r}SO_4Cl$	$^{r}ClSO_4 - {}^{r}SO_4SO_4$

show the relative magnitudes of the correlations between each of the three measures of the concentration of the soil solution and each of the tissue-fluid properties.

The numerical values of all these differences and the probable errors which are necessary for determining their significance may be calculated from the constants in Tables 1 to 3. Since the probable errors of the correlation coefficients are for the most part relatively large because of the generally low value of the coefficients and the relatively small number of determinations upon which they are based, it does not seem desirable to present and discuss in detail the numerical values. The comparisons may be made at a saving of space by a graphic presentation of the differences. (Fig. 4.)

COMPARISON OF CORRELATIONS BETWEEN DIFFERENT TISSUE-FLUID PROPERTIES AND A GIVEN MEASURE OF SOIL SALINITY

In Figure 4 the two coefficients involved are represented on the 18 panels, each of which presents one set of differences. The comparison is between the values of the coefficients given on the lower margin of the panel and that given above the scale representing the depth of the sample. The differences are taken as shown in the first of the two schemes of differences above. The reader will find it convenient to note that the value of the coefficient indicated above the scale of depth is always subtracted from that of the coefficient given on the basal margin of the panel. Thus when the correlation indicated by the marginal symbols has a larger value (algebraically considered, and regarding the reversal of the signs which has been made in the correlations involving soil resistance), the differences (scale of ordinates) will lie above the bar denoting a difference of zero (or, for brevity, the zero bar).

In each of the panels, three scales represent the correlations for the soil samples taken at the first to the fourth foot (1 to 4) and the average correlations (A). The three series of correlations determined are represented by 1, 2–A, and 2–B, as defined on p. 770.

A comparison of the correlations between the electrical resistance of the soil solution and the osmotic concentration (as measured in terms of the freezing-point depression) of the leaf-tissue fluids ($^{r}R\Delta$) with the correlations between soil resistance and the specific electrical conductivity of the leaf-tissue fluids ($^{r}R\kappa$) shows that, for the Egyptian cotton, the differences are practically 0 in series 1 and 2–A. In

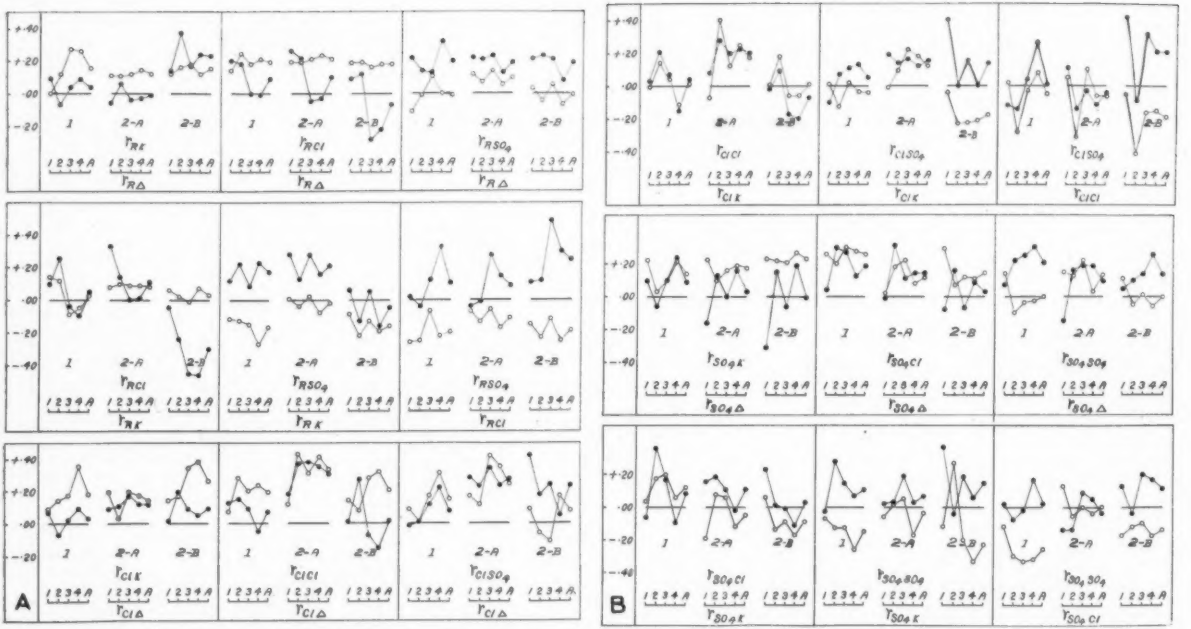

FIGURE 4.—Differences between correlations of various tissue-fluid properties (Series 1, 2–A, and 2–B) with a given measure of soil salinity in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23)

series 2–B the Egyptian plants show higher correlations between soil resistance and osmotic concentration than between soil resistance and electrical conductivity ($^{r}R\Delta > {}^{r}R\kappa$). For the upland cotton, the correlation between soil resistance and osmotic concentration is higher than that between soil resistance and electrical conductivity in all cases ($^{r}R\Delta > {}^{r}R\kappa$).

The correlations between soil resistance and the concentration of all solutes (as measured by the freezing-point depression) in the leaf-tissue fluids are, for upland cotton, higher in all cases than the correlations between soil resistance and the concentration of chlorides in the leaf-tissue fluids ($^{r}R\Delta > {}^{r}RCl$). For the Egyptian cotton the differences are in general much lower, and certain of them are well on the negative side ($^{r}RSO_4 > {}^{r}R\Delta$).

The correlations between soil resistance and total solutes are higher in the Egyptian cotton than those between soil resistance and tissue-fluid sulphates ($^{r}R\Delta > {}^{r}RSO_4$). The differences between these two correlations are smaller in the upland variety and sometimes on the negative side ($^{r}R\Delta_4 > {}^{r}RSO_4$).

In the upland variety $^{r}R\kappa > {}^{r}RCl$ in the majority of cases. The differences are, however, so small that little stress is to be laid on them. In series 2–B the values of ^{r}RCl for Egyptian cotton seem to be distinctly larger than those of $^{r}R\kappa$.

It appears that in some cases $^{r}R\kappa > {}^{r}RSO_4$, whereas in other cases $^{r}R\kappa < {}^{r}RSO_4$ in both Egyptian and upland cotton.

The third panel of the central row of Figure 4, A, shows that in the Egyptian variety the correlation between soil resistance and tissue-fluid chlorides is generally greater than that between soil resistance and tissue-fluid sulphates ($^{r}RCl > {}^{r}RSO_4$), whereas in the upland variety the reverse ($^{r}RSO_4 > {}^{r}RCl$) is invariably true.

The three lower panels of Figure 4, A, and the three upper panels of Figure 4, B, give the results of comparisons of the correlations between the chloride content of the soil and each of the properties of the leaf-tissue fluids.

A comparison of the correlation of the chloride content of the soil and the total solutes of the leaf-tissue fluids as measured in terms of freezing-point depression, $^{r}Cl\Delta$, and the chloride content of the soil and the specific electrical conductivity of the leaf-tissue fluids, $^{r}Cl\kappa$, shows that, in general, in both Egyptian and upland cotton $^{r}Cl\Delta > {}^{r}Cl\kappa$.

These results are substantiated in a general way by the comparisons of the correlations between the chloride content of the soil and the total solutes of the leaf-tissue fluids ($^{r}Cl\Delta$) with those between the chloride content of the soil and the concentration of chlorides ($^{r}ClCl$) and sulphates ($^{r}ClSO_4$) in the leaf-tissue fluids, as shown by the lower central and right-hand panels of Figure 4, A. Generally, although not invariably, $^{r}Cl\Delta > {}^{r}ClCl$ and $^{r}Cl\Delta > {}^{r}ClSO_4$ in both Egyptian and upland cotton.

A comparison of the correlations between the chloride content of the soil and the electrical resistance of the leaf-tissue fluids, $^{r}Cl\kappa$, and the correlations between the chloride content of the soil and the chloride content of the leaf-tissue fluids, $^{r}ClCl$ (fig. 4, B), shows such irregularities that any final conclusion is impossible. There is, however, some evidence that in both the Egyptian and upland varieties

$r Cl\kappa > r ClCl$. In the comparison between $r Cl\kappa$ and $r ClSO_4$ it appears that in general $r Cl\kappa > r ClSO_4$ in the Egyptian variety, whereas the differences are insignificant in the upland variety. In the comparison of $r ClSO_4$ with $r ClCl$, the differences are so evenly distributed about 0 that no final conclusion is to be drawn concerning any differences in these coefficients.

Comparisons of the correlations between the sulphate content of the soil and each of the various tissue-fluid properties of the plant are made in the six lower panels of Figure 4, B. The results from a comparison of $r SO_4\Delta$ with the other tissue-fluid properties show that in all cases $r SO_4\Delta > r SO_4\kappa$ for the upland variety. This condition is not realized in the case of the Egyptian type. In the upland varieties the relation $r SO_4\Delta > r SO_4Cl$ is found in all cases. In the Egyptian type this condition is generally but not invariably realized. Finally the relation $r SO_4\Delta > r SO_4SO_4$ is found in all but one case in the Egyptian type. This condition is not realized in the upland type.

The comparison between $r SO_4\kappa$, on the one hand, and $r SO_4Cl$ and $r SO_4SO_4$ on the other, and between $r SO_4Cl$ and $r SO_4SO_4$ shows results too irregular to merit further discussion until additional data are available.

Notwithstanding various irregularities (believed to be due primarily to limitations in the experiment, which will be discussed later), there seem to be certain definite trends in the results which are important in confirming earlier results and in suggesting further lines of work.

(1) In the upland variety the correlation between the electrical resistance of the soil and the concentration of all solutes of the leaf-tissue fluids (as measured by freezing-point depression) is higher than the correlation between the salinity of the soil and the conductivity of the tissue fluids. The relationship is not so marked in the Egyptian type.

(2) In general the correlation between the analytically determined concentration of the two ions studied in the soils and the total solutes of the tissue fluids as measured in terms of osmotic concentration is higher than that between these ions and the various measures of electrolyte content of the tissue fluids.

(3) In the upland variety the correlations between the soil resistance and total solutes of the tissue fluids are higher than those between soil resistance and the chlorides of the tissue fluids ($r R\Delta > r RCl$), whereas in the Egyptian variety the reverse ($r RCl > r R\Delta$) may be true.

(4) In the Egyptian variety the correlations between soil resistance and total solutes are higher than those between soil resistance and tissue-fluid sulphate content ($r R\Delta > r RSO_4$). The differences between these two correlations are smaller in the upland type, and their sign may in some cases be reversed ($r RSO_4 > r R\Delta$).

(5) Paragraphs 3 and 4 suggest that the correlations between soil salinity and chloride content are higher in the Egyptian variety, whereas the correlations between soil resistance and sulphate content are higher in the upland type. Thus, there seem to be differences between the relationships of the specific ions of the leaf-tissue fluids of Egyptian and upland cotton to the concentration of the electrolytes in the soil as measured in terms of the electrical resistance.

COMPARISON OF CORRELATIONS BETWEEN DIFFERENT MEASURES OF SOIL SALINITY AND A GIVEN TISSUE-FLUID PROPERTY

The second group of comparisons—those showing the differences in the correlations between a given constituent of the leaf-tissue fluids and different measures of soil salinity—are represented graphically in Figure 5, which is constructed on the same principle as Figure 4.

The differences are so taken that when the correlation between the constants given at the lower margin of the panel is higher than that between the constants given above the scale for soil depth, the difference will lie above the bars denoting zero.[5]

Considering all the differences shown in the 12 panels of Figure 5, it appears that they are about equally distributed above and below the zero bars. Thus it must be recognized at the outset that there are no great differences between these pairs of correlations.

An examination of the correlations between soil resistance and tissue-fluid properties in comparison with those between soil chloride content and tissue-fluid properties shows that for freezing-point depression (Δ), specific electrical conductivity (κ), chloride content (Cl), and sulphate content (SO_4), the correlation between soil resistance and the given tissue-fluid property is sometimes higher and sometimes lower than that between soil chloride content and the same tissue-fluid property. A comparison of the correlations between the resistance of the soil and the tissue-fluid properties with the correlations between the sulphate content of the soil and the tissue-fluid properties shows similar results.

Thus it is quite clear that so far as the data of this investigation may serve as a basis for generalization, the electrical resistance of the soil is about as satisfactory a measure of salinity as analytically determined values of either sulphate or chloride content.

Comparison of the correlations between the tissue-fluid properties and the chloride and sulphate contents of the soil shows that for the relationships as a whole the differences are sometimes on the positive and sometimes on the negative side of 0. In short, the tissue-fluid properties are in some cases more closely correlated with the chloride content and in other cases more closely correlated with the sulphate content of the soil.

Since these latter differences are of particular interest because of the fact that the absorption of chlorides and sulphates has been shown to be differential for the two varieties, they may be examined in somewhat greater detail. The upper right-hand panel of Figure 5, A, shows that in upland cotton the correlation between the chlorides of the soil and the freezing-point depression of the tissue fluids is generally higher than that between the sulphate content of the soil and the freezing-point depression of the tissue fluids. The reverse relationship is, however, found in the first series of determination for Egyptian cotton. For the relationship between the chloride and sulphate content of the soil and the electrical conductivity of the tissue fluids, $r Cl\kappa > r SO_4\kappa$ in all but one case in the upland variety. There are, however, many exceptions to this rule in the case of the Egyptian variety.

The comparisons between $r ClCl$ and $r SO_4Cl$ and between $r ClSO_4$ and $r SO_4SO_4$, as shown in the two right-hand panels of the lower

[5] In all cases in which electrical resistance serves as a measure of the concentration of the soil solution the signs of these coefficients have been changed before determining differences.

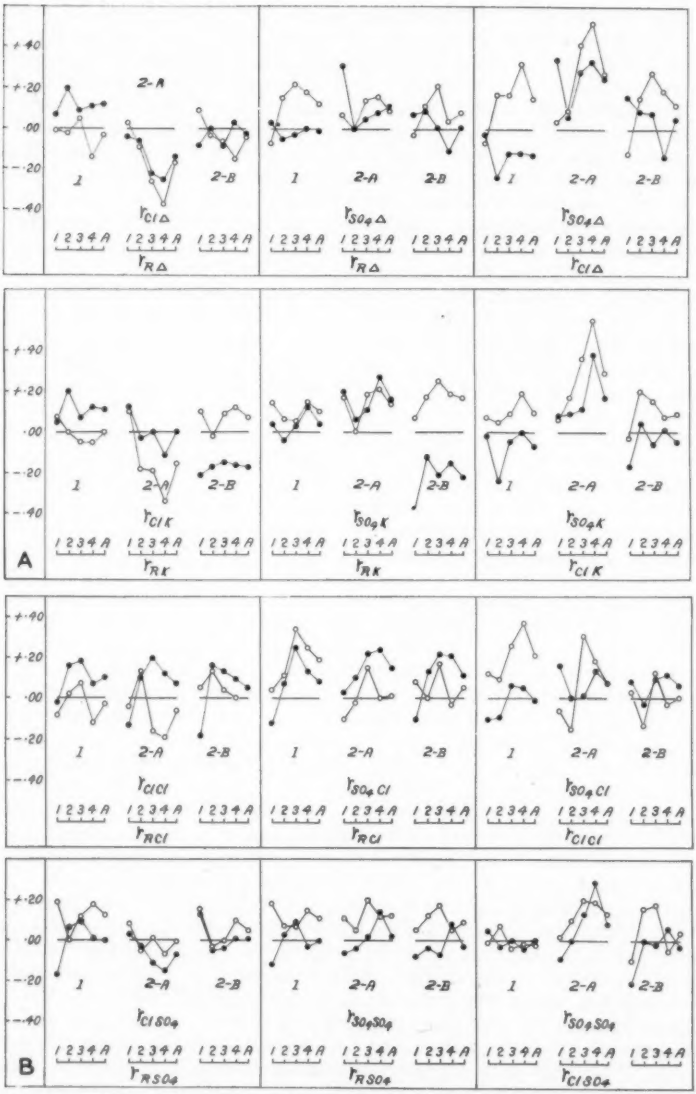

FIGURE 5.—Differences between correlations of a given tissue-fluid property (series 1, 2-A, and 2-B) with various measures of soil salinity in Pima Egyptian cotton (represented by dots) and Lone Star upland cotton (represented by circles) grown at the United States Field Station, Sacaton, Ariz., in 1923 (experiment 1/23)

half of Figure 5, B, are of particular interest because they show that there are no conspicuous and universally constant differences between these pairs of correlations.

DISCUSSION

The results of the present investigation show that there is in general a positive correlation between the salinity of the soil and the osmotic concentration as measured in terms of freezing-point depression (Δ), specific electrical conductivity (κ), chloride content (Cl), and sulphate content (SO_4), of the leaf-tissue fluids of both Pima Egyptian and Lone Star upland cotton.

These relationships prevail whether soil salinity is measured in terms of electrical resistance (in which case the signs of the coefficients have been reversed, since salinity is, within limits, inversely proportional to electrical resistance) or as chloride content or sulphate content expressed as percentages of the air-dry weight of the soil.

Except for the lower numerical values of the coefficients, the results obtained in the present study fully confirm those of the earlier investigation on the tissue fluids of Pima Egyptian and Acala and Meade upland cotton (*6*). The writers are inclined to attribute the lower values of the coefficients obtained in the present investigation (based on experiment 1/23) as compared with the first study (based on experiment 3/22) to the lower technical quality of the data of the present series. This pertains especially to the manner in which the soil samples were taken. The limitations of the methods of sampling employed in experiment 1/23 have been adequately discussed in a study of the relationship between soil salinity and seedling stand (*8*), in which it was found that lower correlations prevailed in the experiment of 1923 than in that of 1922. The explanation of the lower numerical values of the coefficients is probably the same for seedling stand and for tissue-fluid properties.

The present results extend those already available by showing that the relationships between the properties of the soil and the characteristics of the plant hold for the analytically determined solutes (chlorides and sulphates) of the soil as well as for the more general measure of soil salinity provided by the electrical resistance of the soil mass.

Taking these results as a whole, it appears that the present data show no regular and universally valid difference between the correlations of any of the three measures of soil salinity and the tissue-fluid properties. There is unquestionably some correlation between each of these three measures of soil salinity and the various measures of concentration of solutes in the leaf-tissue fluids. It is not possible to assert, on the basis of the present results, that the correlations are universally higher for one measure of soil salinity than for another.

In any consideration of these results, the probability that the concentrations of the different salts in the soil are not independent but correlated must be borne in mind. This fact must have its bearing on the relationship between the electrical resistance of the soil and the tissue-fluid properties of the plant. Since electrical resistance depends upon all ions, it is not unreasonable to assume that it might give a higher correlation with tissue-fluid properties depending upon a number of solutes than would any one ion of the soil solution. This problem requires further investigation.

The foregoing explanation of the fact that the correlation between the electrical resistance of the soil and the tissue-fluid properties is equal to or greater than that between the specific ions of the soil and the tissue-fluid properties applies only to such general measures of tissue-fluid concentration as freezing-point depression and specific electrical conductivity. It can not be urged for such relations as those between the chloride content of the soil and the chloride content of the tissue fluids and between the sulphate content of the soil and the sulphate content of the tissue fluids in which other factors must be taken into consideration.

It is quite possible that the method of expressing the concentration of chloride and sulphate as a percentage of the dry weight of the soil, while conventional, is not the most advantageous for purposes of this kind. It would unquestionably be better if some measure of the concentration of these ions in the soil solution were made the basis of the correlations. Furthermore, it must be borne in mind that there may be large reserves of sulphate in the soil in the form of the relatively insoluble calcium sulphate. Thus the concentration of sulphate at any one moment in a water extract, expressed as a percentage of the weight of the soil, would not necessarily represent the conditions to which the plant itself is subjected.

It will be clear from the foregoing considerations that the problem of the relationship between the soil and the plant as it grows on the ordinary agricultural field is one of very great complexity. In the investigation of these interrelationships the correlations between the concentrations of the various constituents of the soils complicates the problem. More refined methods (both experimental and statistical) and larger series of data for both the soil and the plant will be necessary to unravel fully the tangle of interrelationships. Because of these obvious difficulties it seems best to defer a further statistical study of the problem until better data than those now in hand are available.

While the results secured up to the present time have an important bearing on the physiology of the cotton plant, they have a greater importance in relation to the methodology of agricultural investigation, in that they show that by a proper combination of agronomic, chemical, and biometric methods it will be possible to investigate in the field many problems which have been assumed to be open to investigation only under the controlled conditions of the laboratory.

The criticism may be made that the correlations in the present paper are of too low an order of magnitude to be taken as a trustworthy measure of the relationship between the variables of the soil and the plant. To this objection the reply is threefold.

(1) The assumption is tacitly made that in laboratory experimentation the results are of a high degree of trustworthiness. In the investigation of the relationship of the characters of the plant to the properties of the soil or culture medium this is by no means invariably the case. As far as the writers are aware it is still to be demonstrated that in the investigation of the relationship between the concentration of the solution and the characteristics of the plant results of a higher order of precision have been obtained in the laboratory than those which have been secured in these studies.

(2) There is no reason to assume that any individual interrelationship will be of a high order of magnitude. The determination of

the order of magnitude of relationships between soil properties and plant characters is, indeed, one of the objects of any investigation of this kind. It is the trustworthiness rather than the magnitude of the constants which is of importance in determining the value of the results.

(3) Various reasons for believing that the correlations for the present experiment were far from ideal have been indicated in the discussion. Much higher coefficients have been obtained for certain of the variables in an earlier investigation (*6*), which is believed to be technically better. Both this and the present study have been reconnaissance experiments. There is every reason to believe that the organization and technical details of such experiments can be materially improved, with the result that higher correlations and better fits of the regression lines or curves will be obtained.

SUMMARY

The present paper is one of a series dealing with the measurement of the relationships between the properties of the soil and the characteristics of the crop plant produced (*6*, *8*, *9*).

When suitable data are available the relationships between the charactertistics of the plant and the salinity of the soil may be satisfactorily expressed in terms of the correlation coefficients. While salinity is an advantageous soil property for a first investigation, there is every reason to believe that the method may be extended to other soil properties.

The present paper confirms and extends the findings of the first study (*6*) of the relationship between the salinity of the soil and the tissue-fluid properties of Pima Egyptian, Meade upland, and Acala upland cotton in that positive correlations are demonstrated between the electrical conductivity, the chloride content, and the sulphate content of the soil, on the one hand, and the freezing-point depression, specific electrical conductivity, and chloride and sulphate content of the leaf-tissue fluids of the plant on the other. The coefficients obtained in the present study are lower than those found in the first investigation. The reasons for the differences are discussed.

The present findings extend those of the earlier investigation in that they are based on two additional variables for the soil (chloride content and sulphate content) and on an additional variable for the tissue fluids (sulphate content). The fact that statistically significant correlations have been obtained for all the combinations of the variables of the soil and the plant demonstrates the wide usefulness of the method of research employed.

The present study is admittedly preliminary in nature. It has fulfilled its purpose by confirming and extending previous results and by showing that physiological investigations of this kind may be carried out in conjunction with ordinary field-plot studies. When the refinements of technic that may be made at many points are available, results of far greater exactness and wider significance may be expected.

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NOTES ON THE HISTOLOGY OF THE ALMOND[1]

By V. A. PEASE

Associate Microscopist, Food Research Division, Chemical and Technological Research, Bureau of Chemistry and Soils, United States Department of Agriculture

INTRODUCTION

In connection with a study of one of the technological problems involved in the salting of domestic almonds, some rather interesting data on the histology of the almond kernel or seed have been assembled. This material is presented here not only on account of its general interest to histologists, but because it may afford some assistance to the trade in developing a method for the determinination of types if not of varieties of shelled almonds as they appear in the open market.

MATERIAL

The material studied[2] consists of four varieties of domestic almonds and four varieties of imported almonds. The domestic almonds, Nonpareil, Ne Plus Ultra, I. X. L., and Drake, are among the leading varieties grown in California, and the imported nuts, Etna, Marcona, Valencia, and Alicante, originally brought from Italy and Spain, are largely used by bakers and confectioners. The domestic nuts are paper and soft shelled, while the imported nuts are standard and hard shelled. On the basis of the hardness of the shell, these samples would be classed as follows:[3]

Paper	I. X. L.
	Ne Plus Ultra.
	Nonpareil.
Soft	Drake.
Standard	Marcona.
Hard	Valencia.
	Etna.
	Alicante (?).

The almond fruit is a typical drupe; that is, it consists of a single seed surrounded by a stony endocarp which forms the shell, a fleshy mesocarp, and an outer skin or epicarp. The mesocarp is tough and leathery and, with its protecting epicarp, splits away from the endocarp at maturity. The difference between hard and soft shelled almonds lies in the difference in texture of the endocarp. The shelled almond, or kernel, consists of the embryo, surrounded by the modified tissues of the ovule. The embryo itself is made up of a small, pointed radicle, a delicate plumule, and two straight massive cotyledons whose cells are closely packed with reserve food material in the form of oil droplets and protein granules. The surrounding tissues consist of the remnants of the endosperm and nucellus, and the integuments.

[1] Received for publication July 29, 1930, issued December, 1930.

[2] This material was furnished by the California Almond Growers' Exchange to whom the writer is greatly indebted.

[3] Based on General Information on Almonds, Foodstuffs Division, Bureau of Foreign and Domestic Commerce, U. S. Department of Commerce, 1928. [Mimeographed.]

REVIEW OF LITERATURE

Early work on the structure of the almond was done with a view to distinguishing microscopically between the kernels of almonds and such related seeds as peaches, apricots, and plums, which were being substituted for almonds, both in almond paste and in the expressing of the oil. Early workers on the microscopy of the almond seed agreed that there were no differences in structure between bitter and sweet almonds (*1*).[4] The question of a difference between hard and soft shelled almonds seems not to have been raised until Young (*13*) contrasted them. His opening statement, however, is, "The bitter almond and the hard and soft or paper shelled varieties of the sweet almond all belong to one species and are hence very similar in structure." Young's work is the most detailed histological study of the almond published in English. Meyer (*6*) gives a careful description of the development of the seed from fertilization to maturity, with a discussion of the various tissues in the ripened seed and seed coat. Tschirch (*10*) bases his discussion on the best of the earlier workers and adds several new points, especially along the line of microchemical tests for cell-wall substances. Kraemer (*5*) offers probably the best summary in English of later foreign work, with well-executed line drawings, which are for the most part adaptations from other writers. Winton (*11*) seems to be the only other American writer who has published work on this subject.

METHOD

Preliminary examination having shown that in all probability the greatest anatomical differences between varieties or types of nuts were to be found in the "skin" or tissues surrounding the embryo, the structure of these tissues was worked out in some detail. Skins removed by blanching, or soaking for two or three minutes in boiling water, were boiled in 2 per cent potassium hydroxide and allowed to stand overnight, or until shrunken tissues and "obliterated cells" were sufficiently swelled so that their shape, size, arrangement, and relationships could be determined. Following this treatment, the aleurone layer could be stripped from the inner surface of the testa, the epidermal stone cells or hairs could be scraped from the outer surface with a dull scalpel, and the remaining tissues could be teased apart with needles or fine-pointed tweezers. Material was also embedded in paraffin, sectioned 10 microns thick, and stained with safranine and light green, using Land's schedule as given by Chamberlain (*2*). In addition to differentiating cellulose and lignin in the testa, this stain proved particularly satisfactory for the storage tissue of the cotyledons, the cell walls staining green and the protein granules bright red. Microchemical tests used were: For lignin, phloroglucinol-hydrochloric acid; for cellulose, chlorzinciodide; for cutin and oil, Sudan III; for tannin, ferric chloride; for protein, iodine in potassium iodide and the xanthoproteic reaction.

THE SEED COAT

The inner white layer stripped away from the brown or yellowish-brown testa after maceration in 2 per cent potassium hydroxide con-

[4] References made by number (italic) to Literature Cited, p. 800.

sists almost entirely of aleurone and obliterated cells; that is, endosperm and nucellus or perisperm. The aleurone cells of the endosperm (fig. 1, A, 2, *al*) are thick walled, quadrilateral to hexagonal

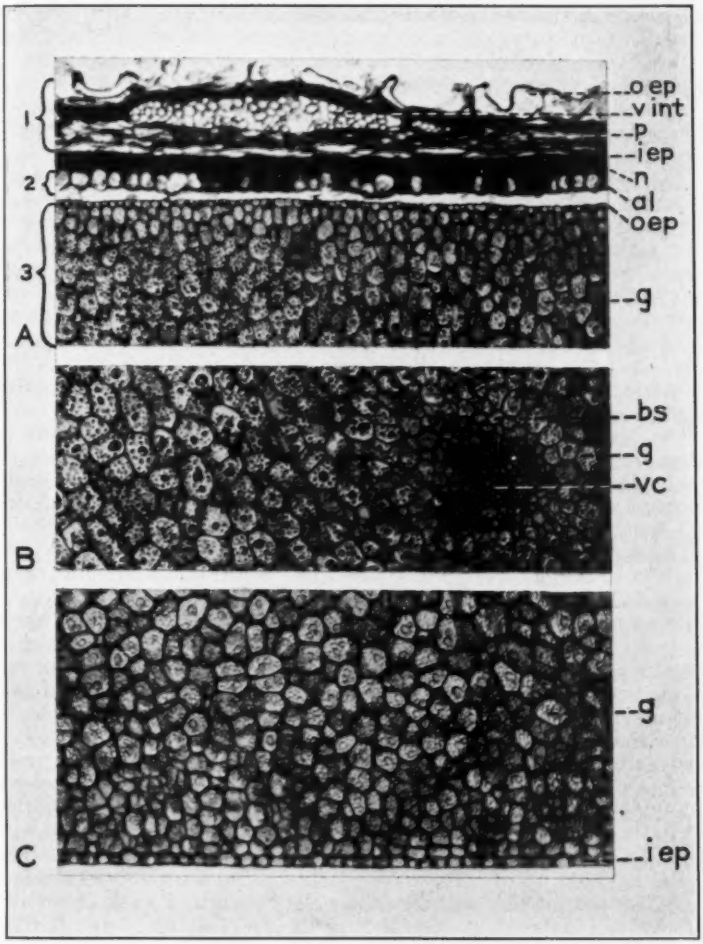

FIGURE 1.—Cross sections of Nonpareil almond. × 145. A, Testa and outer portion of cotyledon: 1, Integuments: *o ep*, Outer epidermis; *p*, flattened cells of parenchyma; *v int*, vascular bundle; *i ep*, inner epidermis; 2, nucellus and endosperm; *n*, obliterated tissue of nucellus; *al*, aleurone layer; 3, cotyledon: *o ep*, outer, or lower, epidermis; *g*, ground tissue with proteing ranules. B, Central portion of cotyledon: *g*, Ground tissue; *b s*, bundle sheath; *v c*, vascular bundle. C, Inner portion of cotyledon: *g*, Ground tissue; *i ep*, inner, or upper, epidermis

in surface outline, usually somewhat elongated in the direction of the long axis of the seed, and without intercellular spaces. Each cell contains one or more large oil globules and numerous fine protein granules. The radicle is embedded in a mass of aleurone cells, but over the flat surface of the cotyledons the layer is usually one cell

thick, although it may be two or even three cells thick. The thickened areas may be due to tangential division of the cells, but in some instances they seem to be due to infoldings, the folds being parallel to the long axis of the seed. Individual aleurone cells vary in size and shape in different regions and in different varieties of nuts, but these differences are not significant in the determination of varieties. A thin layer of obliterated cells can sometimes be found between the two cotyledons or on the inner surface of the aleurone layer. This is composed of fragmentary traces of thin-walled endosperm tissue. It is especially evident in the chalazal region. Its presence between the cotyledons was first noted and figured by Hartwich (*4*).

The perisperm or nucellus (fig. 1, A, 2, *n*) consists of obliterated tissue, in which the individual cells are faintly visible after prolonged treatment with potassium hydroxide. They are very large, roughly 150 by 400 microns, with extremely delicate colorless walls. The outermost layer or epidermis of the nucellus consists of narrow, elongated cells with faintly beaded walls, regularly arranged with their long diameters parallel with the long axis of the seed. They are approximately 50 by 150 microns, although varying widely from these figures, which are the average of 125 measurements on four varieties, two domestic and two foreign. The variation in a single piece of tissue is often as great as in the different types of nuts.

The testa, or outer portion of the seed coat, is of integumentary origin. It is this part of the almond and related seeds that has received most attention from students of histology. Several points in structural anatomy have not yet been cleared up, and this still remains a fertile field for study.

The inner epidermis (fig. 1, A, 1, *i ep*) consists of small flat cells, roughly quadrilaterial in surface view, with finely sinuate walls. These cells appear to be cutinized on their outer surface, the layer of cutin separating them from the extremely thin-walled epidermis of the nucellus. This interpretation is contrary to the opinion of Moeller and Thoms (*8*), who state that the cuticularized membrane belongs to the inner tissues which lie in contact with the brown integumentary layer, but is in agreement with that of Péchoutre (*9*), who states that the inner epidermis of the seed coat is slightly thickened and cutinized. The cell content forms a solid platelike mass, highly refractive, dark brown in color, and soluble in hot water and in 2 per cent potassium hydroxide, but insoluble in alcohol and ether.

The body of the testa is made up of flattened parenchyma cells (fig. 1, A, 1, *p*) separated into an inner and outer region. The inner region is made up of cells with colorless cellulose walls. The outer region consists of cells with lignified yellowish-brown walls. Between the two regions are the vascular bundles or veins (fig. 1, A, 1, *v int*) radiating from the chalaza and running practically parallel throughout the length of the seed to the micropylar tip. Along the veins are found rosette crystal aggregates of calcium oxalate, more abundant in hard-shelled types. The parenchyma cells of both the inner and outer regions when swelled in 2 per cent potassium hydroxide form a loose spongy tissue, the individual cells usually being in direct contact only by means of short blunt processes. Hence there is a large amount of intercellular space which is not apparent in the normal flattened condition of the cells in the ripened seed coat. The cells of the exterior subepidermal layers are much smaller, but are also

loosely arranged with much intercellular space. (Fig. 3, A, *c*.) The development of the integuments during the maturing of the seed has not been followed, and no statement appears in the literature consulted as to where the boundary between the two integuments lies. Péchoutre's sketches indicate that the tissues of the inner integument are more collapsed than those of the outer. It is natural to assume that the inner cells with cellulose walls are morphologically a part of the inner integument and the outer lignified cells are a part of the outer integument.

The outer epidermal layer of the testa, which forms the surface of the seed, has been subjected to profound modification. Most of the cells are enormously enlarged, and in many the walls are thickened and lignified. (Figs. 2, 3, and 4.[5]) The phloroglucinol-hydrochloric acid reaction shows the distribution and relative abundance of the lignified cells very clearly. (Fig. 4, B.) These lignified epidermal cells have attracted the attention of histologists for many years. They have been variously described as hair cells, barrel cells (Tonnenzellen), or balloon cells (Kugelzellen), but are usually spoken of as stone cells. They are described by the early writers as egg-shaped or globular, though Young (*13*) figures extremely elongated cells, and Winton (*11*) states that they are commonly higher than broad and often elongated, like trichomes. In fact, there has been some discussion as to whether they are to be considered as epidermis or as trichomes.

The cell lumen is empty, but a thin protoplasmic layer is closely appressed to the inner surface of the cell wall. This is apparently what Tschirch (*10*) considers an inner layer of cell-wall substance, which he differentiates from the lignified portion of the wall. This layer gives a deep-blue reaction with ferric chloride, indicating the presence of tannin.

The epidermal cells vary noticeably in size, shape, degree of lignification, and distribution of lignified cells (figs. 2, 3, and 4), not only as between hard and soft shelled almonds, which has been noted by several writers, but also as between the varieties in either class, a fact which seems to have escaped the notice of earlier observers. In fact, Berg (*1*) states that there are no differences in structure between sweet and bitter almonds, the only difference between the two being in the cell contents of the kernels. Young (*13*) describes and figures the stone cells of the epidermis of the integuments of hard-shelled almonds, but says that in soft-shelled almonds a few of the epidermal cells have thicker walls than others, suggesting the stone cells found in the epidermis of the hard-shelled nut. Winton (*11*) agrees with Young. No varietal comparisons seem to have been made.

Wittmack and Buchwald (*12*) seem to have observed only the lignified cells and describe them as forming a discontinuous layer in the ripe seed. Hannig (*3*) first described the thin-walled nonlignified cells which are interspersed with the lignified cells, and remarked that they are sometimes found unaltered, but frequently are collapsed, and usually are completely crushed or altogether destroyed. This, he observed, gives the lignified cells the appearance of standing out from the surface singly or in groups; hence the opinion of earlier writers that they are hairs.

[5] The writer is greatly indebted to G. L. Keenan, microanalyst, Food and Drug Administration, U. S. Department of Agriculture, for assistance in the preparation of these figures.

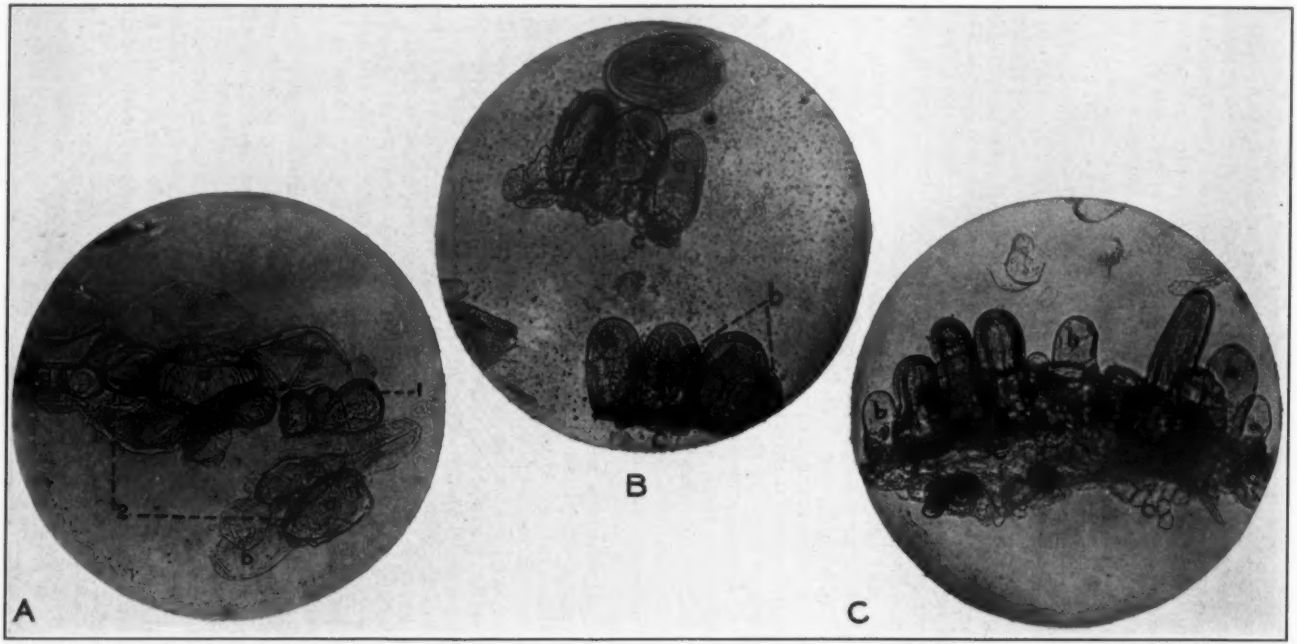

FIGURE 2.—A, Outer epidermis of testa of Nonpareil almond: 1, Group of three stone cells from the side, showing heavy walls and pores; 2, surface view: *a*, Stone cells; *b*, thin-walled cells. B, Epidermal cells of Drake almond: *a*, Stone cells; *b*, thin-walled cells; *c*, subepidermal cells. C, Group of cells from Marcona almond: *a*, Stone cells; *b*, thin-walled cells; *c*, subepidermal cells. Macerated material, unstained. × 80

FIGURE 3.—Etna almond, A, Various types of cells from the testa: *a*, Stone cells of outer epidermis; *b*, thin-walled cells of outer epidermis; *c*, subepidermal cells. B, A group of very large epidermal cells: *a*, Stone cells; *b*, thin-walled cells. Macerated material, unstained. × 80

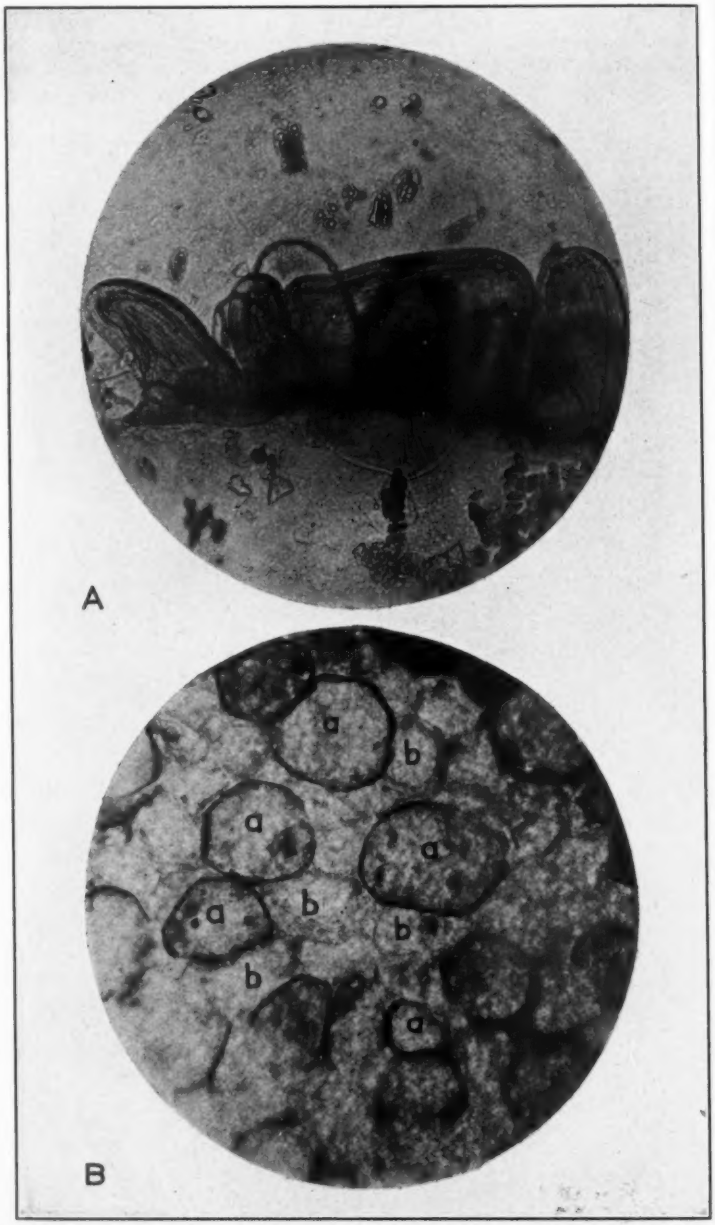

FIGURE 4.—Etna almond. A, Epidermal stone cells, over a vein, showing modified shapes. Macerated tissue, unstained. × 80. B, Surface view of testa: *a*, Stone cell; *b*, thin-walled cell. Stained with phloroglucinol-hydrochloric acid. × 80

The present study shows that in the varieties examined thick-walled lignified cells occur in the epidermal layer of the testa in both soft and hard shelled almonds. The lignified cells do not differ from the unlignified in size or shape, and are distributed irregularly over the surface, being apparently more closely crowded and more elongated over the radicle, and especially along the base of the raphe. Over the veins, and especially along the raphe, they often assume bizarre shapes as if stretched by the increasing growth of the seed. Over the chalaza the cells are much smaller.

The bases of the cells where in contact with each other and with the subepidermal layer are porous, the pores apparently connecting the cavities of adjacent cells. These pores are simple and unbranched, and usually round, but they may be elliptical, and several may be ranged at irregular intervals along shallow crescentic or serpentine grooves in the cell wall.

The free surface of the cell may be slightly domed or enormously extended, smooth on the outer surface and punctate or channeled on the inner. The elongated cells may be balloonlike, nipplelike, or beaked, and many of the individual cells are so large as to be plainly visible through a hand lens. Their rounded extremities give the roughened granular appearance to the shelled nut.

In the varieties of soft-shelled almonds studied the walls of the lignified cells are faintly colored a yellowish brown, and are thickened very little; the pores are comparatively few and aggregated in groups, and the inner surface of the outer wall is not punctate or ridged. (Fig. 2, A and B.) In all four varieties they give a very definite phloroglucinol-hydrochloric acid reaction. In the Nonpareil (fig. 2, A, 1), which appears smooth to the naked eye, the outer wall is only slightly domed, the depth of the cell being approximately equal to its diameter and averaging about 85 microns. In I. X. L. and Drake (fig. 2, B) the cells average approximately 130 microns in height and 100 microns in diameter, although cells were found more than 450 microns in height or diameter, such cells being easily visible with a hand lens. In Ne Plus Ultra the cells average approximately 175 microns in height and 135 microns in diameter, some cells measuring more than 450 microns.

In the hard-shelled almonds the lignified cells have heavily thickened walls, especially the free outer walls, which show stratification in all varieties when swelled in 2 per cent potassium hydroxide. They are strongly colored brown or yellowish brown. The Marcona (fig. 2, C) has the smallest cells measured, averaging approximately 130 microns in height and 75 microns in diameter, although reaching to from 300 to 370 microns in some instances. In the Etna (figs. 3 and 4) occurs the most marked elongation, the cells averaging approximately 300 microns in height and 130 microns in diameter, some cells being 650 microns in height, some 500 microns in width. In Valencia the cells average approximately 240 microns in height and 130 microns in diameter, with some cells exceeding 500 microns in either height or diameter. In Alicante the average measurements approximate 150 microns for height and 100 microns for diameter, although an occasional cell reaches 430 microns in height or 650 microns in diameter, the greatest diameter measured in any of the varieties.

In other words, the ratio of height to width in the four varieties of soft-shelled almonds averages 5:4, whereas in the four varieties of hard-shelled almonds the average ratio is 2:1. It must be borne in mind that extremes in height and diameter are not necessarily found in the same cell. Extremely elongated cells (fig. 3, B) may be found over any portion of the surface, with the exception of the chalaza, and they are especially abundant toward the apical end and along the margins of the raphe. Cells with the diameters stretched parallel to the long axis of the seed (fig. 4, A) are found immediately over the veins, and especially over the raphe. The averages given are based on at least 200 measurements for each variety.

A comparison of figures obtained in this study with those given by earlier workers is of interest. Such a comparison is presented in Tables 1 and 2.

TABLE 1.—*Measurements of lignified cells of integuments of hard and soft shelled almonds*

Variety	Height (μ)			Diameter (μ)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
DOMESTIC ALMONDS, SOFT SHELLED						
Nonpareil	35	163	83	35	185	87
I. X. L.	43	483	131	21	490	97
Drake	28	454	131	21	440	107
Ne Plus Ultra	21	483	177	28	461	136
Average			130			106
IMPORTED ALMONDS, HARD SHELLED						
Marcona	35	319	132	14	369	75
Etna	57	653	310	21	497	133
Valencia	35	547	241	21	516	132
Alicante	43	433	153	21	653	98
Average			209			109

TABLE 2.—*Measurements of lignified cells of integuments of almonds*

[Taken from the literature]

Author	Year	Variety	Height (μ)			Diameter (μ)		
			Minimum	Maximum	Average	Minimum	Maximum	Average
Wittmack & Buchwald (*12*)	1901		90 136	109 159		69	84	100
J. Moeller (*7*)	1905	Italian			175			
E. Hannig (*3*)	1911		64 (—)	144 (200)		23		
A. Tschirch (*10*)	1912		120	335		70	135	
W. J. Young (*13*)	1912	Soft				100	200	
		Hard		400				
A. L. Winton (*11*)	1916	Hard	60	400	100+			
H. Kraemer (*5*)	1928		70	175		65	100	

THE EMBRYO

In the dormant embryo of the ripened seed the massive cotyledons are usually flat and closely appressed, but an occasional seed is found in which one cotyledon is folded on itself longitudinally and the other is wrapped about it more or less completely. In most such cases the inner cotyledon is smaller, and it may be much underdeveloped or possibly even atrophied as a result of pressure from the surrounding cotyledon, which has a distinct advantage so far as expansion due to growth is concerned.

The cells of the ground tissue are fairly large and are packed with stored food material, each cell containing from one to several large protein granules and many small ones (fig. 1, A, B, and C, *g*), in addition to oil and soluble carbohydrates. In some cases there appear to be areas of physiological breakdown in otherwise normal cotyledons, the cells appearing empty and collapsed under the microscope.

The system of venation is well developed, forming a network which is very evident in longitudinal sections. The epidermal cells of the inner and outer surfaces of the cotyledon, corresponding to the upper and lower surfaces of a leaf, respectively, are small and narrow, the long axis of the cell being parallel with the long axis of the cotyledon. The outer or lower epidermal cells are much smaller than the inner and upper ones. Young's photomicrograph (*2*) is apparently a surface view of the outer epidermis. The difference in size of the two epidermal layers is well shown in Figure 1, A, 3, *o ep*, and C, *i ep*. This figure also shows the contrast in size between the cells of the epidermal layers and those of the ground tissue. The cell walls in all embryo cells are unmodified parenchyma and are extremely thin. Modifications extend only to size, shape, and content, with the exception of the beginning of thickening in the tracheal tissue of the veins.

SUMMARY AND CONCLUSIONS

A comparative study of the histology of four varieties of domestic soft-shelled almonds and four imported hard-shelled varieties is reported.

Evidence is presented to show that the chief difference between the two types of nuts lies in the structure of the cells of the outer epidermis of the testa, or the surface of the seed coat.

The modified epidermal cells of soft-shelled almonds are lignified, but only slightly as compared with those of hard-shelled almonds. They can therefore be considered as poorly developed stone cells.

Careful measurements indicate that in addition to being heavier walled the stone cells of the seed coat of hard-shelled almonds are more elongated than those of soft-shelled almonds, the ratio of length to width in hard-shelled almonds being approximately 2 to 1 and in soft-shelled almonds 5 to 4.

The importance of this study of the variation in the surface cells of the testa lies in the fact that with further examination and correlation of data it may be possible to develop an easily workable method for distinguishing the different varieties of almonds in the shelled condition, thus detecting or preventing misrepresentation or adulteration.

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THE ASPERGILLI AND THEIR RELATION TO DECAY IN APPLES[1]

By GLENN A. HUBER[2]

Fellow in Plant Pathology, Washington Agricultural Experiment Station

INTRODUCTION

For the past three years investigations have been in progress at the Agricultural Experiment Station of the State College of Washington to determine the nature and identity of the fungi that cause decay of apples in cold storage. Many different organisms have been isolated and their pathogenicity determined by inoculation and reisolation. As a part of this work a study of the fungous flora of the normal apple has also been made.

WASHING EXPERIMENTS

In the investigation of the fungous flora of the normal apple the fruits used for washing were secured from the apple-growing districts of Wenatchee, Wash. Those used in 1927 were sorted and packed at Wenatchee and then shipped to Pullman, where they were placed in cold storage. In 1928, however, the apples were wrapped in sterile wraps as soon as they were picked in order to prevent spores from collecting on them while they were going through the processes of sorting and packing. They were then carefully packed and shipped to Pullman and placed in cold storage.

The apples were kept in cold storage until they were taken to the laboratory, where they were immediately washed. In order to prevent contamination in transferring the fruit from storage to the laboratory, each apple was handled in such a way as not to disturb the wraps.

The walls and working table of the culture room were washed with $HgCl_2$, 1–500, and the air was thoroughly atomized with a similar solution before the washing of each set of apples.

Each apple was carefully unwrapped and placed in a sterile damp chamber which contained 100 c. c. of sterile water. The lid of the damp chamber was raised only high enough to permit the handling of the apple. The fruit was held firmly with sterile forceps and thoroughly scrubbed with a sterile, ½-inch, stiff stencil brush for five minutes. Plates were made in triplicate with one-eighth, one-fourth, and one-half cubic centimeter, respectively, from the thoroughly agitated wash water by removing the suspension to sterile Petri dishes with 1 c. c. sterile pipettes, and then pouring the medium. The medium used was potato agar containing 2 per cent dextrose. To inhibit bacterial growth 1 drop of 25 per cent lactic acid was added to each tube of medium before it was poured. The suspension and medium were

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[2] The writer wishes to express his indebtedness to Dr. F. D. Heald, under whoes direction this study was made, for assistance and inspiration throughout the work; and to Dr. L. K. Jones for helpful suggestions from time to time during the progress of the work this past season.

thoroughly mixed by a circular motion and the plates allowed to harden. They were then incubated in the laboratory at ordinary room temperature, and after three days the number and the type of colonies were recorded. Isolations were made from the various colonies, and stock cultures of each were stored in the low-temperature chest for later identification.

After several sets of apples had been washed, it was decided to find how many spores remained on the fruit after washing. Sartory and Fillassier [3] had found that a large number of spores remained on the surface of fruit after the first washing, but that the number was greatly reduced by a second washing and still further reduced by a third.

After an apple had been thoroughly washed for five minutes, as described above, it was rinsed in sterile water and thoroughly washed a second time for five minutes in 100 c. c. sterile water as before. It was again rinsed in sterile water and washed a third time. The waters from the first, second, and third washings were plated out by the method described above and a count of the number of colonies produced from each washing was taken after three days' incubation at room temperature. A number of apples (125 size) were tested in this way. The results of these tests are given in Table 1.

The results here shown are very similar to those obtained by Sartory and Fillassier. All the spores were not removed by the first washing, but the number was greatly reduced by a second and a third washing.

TABLE 1.—*Number of spores (colony counts) per apple obtained in successive washings of normal sound fruit*

[Apples 1–6, wrapped in orchard immediately after picking; apples 7–9, storage apples]

Apple No.	Number of spores in—			Total number of spores	Apple No.	Number of spores in—			Total number of spores
	First washing	Second washing	Third washing			First washing	Second washing	Third washing	
1	108,300	29,000	13,400	150,700	6	11,100	2,200	700	14,000
2	31,800	1,300	1,000	34,100	7	138,400	82,700	13,200	234,300
3	55,600	44,600	12,400	112,600	8	146,700	82,000	42,900	271,600
4	60,300	37,300	14,400	112,000	9	108,000	47,800	9,200	165,000
5	108,000	32,000	19,100	159,100					

DESCRIPTION OF FORMS

Thom and Church [4] have made a careful study of the Aspergilli, and their nomenclature is followed in this paper.

The cultures on which the following descriptions were based were incubated in the laboratory at room tempearture and observations were made throughout the entire growth of the colonies. Single-spore cultures growing on Czapeks' solution agar [5] as described by Thom and Church were used in all cases. Some of the forms have been definitely assigned to a given species, while the identification of other forms is so uncertain that they have been placed in the nearest form groups.

[3] SARTORY, A., and FILLASSIER, A. LES FRUITS PORTEURS DE MICROBES. Compt. Rend. Soc. Biol. [Paris] (Année 61, t. 2) 67: 445–447. 1909.

[4] THOM, C., and CHURCH, M. B. THE ASPERGILLI. 272 p., illus. Baltimore. 1926.

[5] Czapeks' solution agar: Water 1,000 c. c.; sodium nitrate 3 gm.; potassium phosphate (K_2HPO_4) 1 gm.; magnesium sulphate 0.5 gm.; potassium chloride 0.5 gm.; ferrous sulphate 0.01 gm.; sucrose 30 gm.; agar-agar 15 gm.

All the forms were washed from normal Jonathan apples, Nos. 1 to 5, from the 1927 crop and Nos. 6 to 11 from the 1928 crop.

FORM NO. 1

Form 1 (fig. 1) belongs to the *Aspergillus ochraceus* group, *sulphureus* series, but does not agree in all respects with any of the species described by Thom and Church.

Colonies pale yellow, close to Naples yellow (Ridgway, Pl. XVI),[6] growth regular; reverse at first white with yellowing center, becoming reddish brown in old cultures. Conidial heads globose, up to 200μ in diameter, mostly 160μ. Stalks rising from submerged hyphae, up to 1,500μ in length and up to 16μ in diameter, walls light yellow, pitted; vesicle globose, up to 60μ in diameter; sterigmata in two series, primary sterigmata about twice as long as the secondary and closely

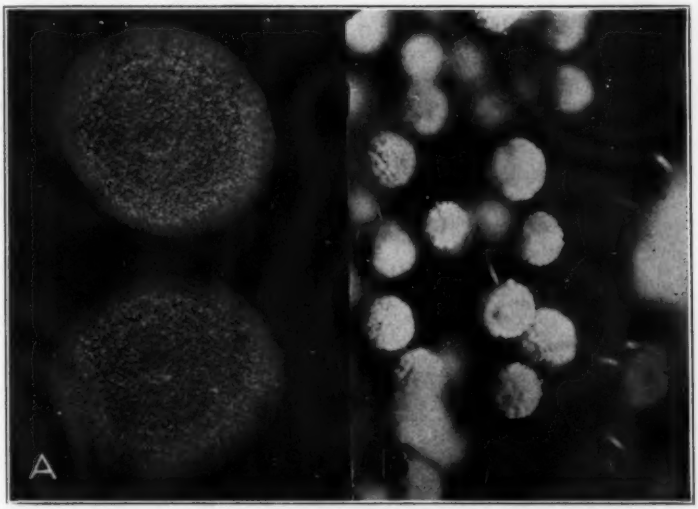

FIGURE 1.—Aspergillus form No. 1. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

packed to form a radiate head; conidia globose to subglobose, 2μ to 3.5μ in diameter. Perithecia not found, sclerotia purple, globose, up to 800μ in diameter, single, scattered over entire colony.

FORM NO. 2

Form 2 (fig. 2) is easily placed in the *Aspergillus tamarii* group, and is practically identical with *A. tamarii*.

The vegetative hyphae are mostly aerial, white, and more or less cottony. The fruiting areas are pale yellow at first, soon becoming a deep yellow, then passing through the orange-yellows to a dark brown in old cultures; reverse cream colored at first, becoming light brown with age. Conidial heads more or less globose when young, soon becoming very loose, the conidial chains diverging in all directions, up to 500μ in diameter with radiating chains or columns of conidia. Stalks apparently smooth, slightly pitted in some cases, up to 1,500μ in length and up to 16μ in diameter; vesicles globose, up to 75μ in diameter; sterigmata one series, up to 40μ long in larger heads and up to 4μ wide; conidia globose to pyriform, tuberculate, 4μ to 6μ in diameter. Perithecia none. Sclerotia none.

[6] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

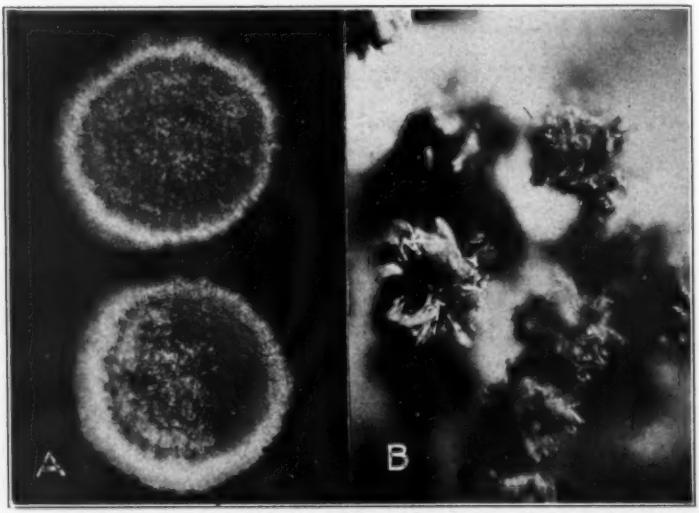

FIGURE 2.—Aspergillus form No. 2. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

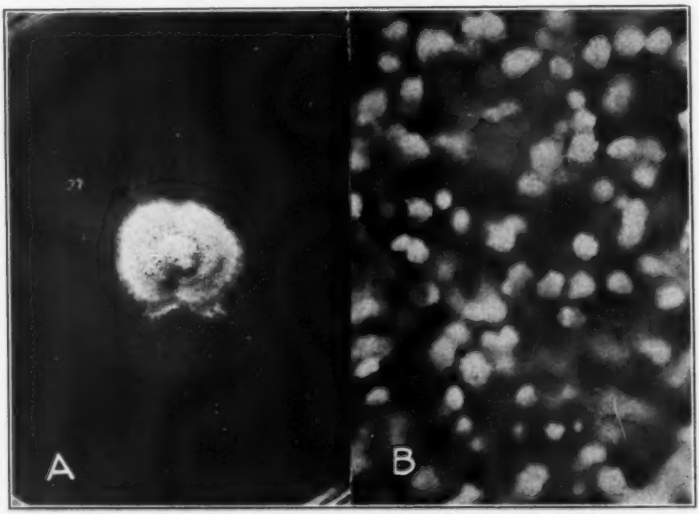

FIGURE 3.—Aspergillus form No. 3. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

FORM NO. 3

Form 3 (fig. 3) is placed in the white-spored Aspergillus group, but further identification has not been made.

Colony white at first, turning to a pale pink with a tinge of yellow around the edge in old cultures; reverse yellow to greenish orange. Conidial heads varying greatly in size and shape in the same colony, from hemispherical to columnar, stalks smooth, up to 250μ in length and up to 6μ in width, colorless, sparingly septate; vesicles more or less flask-shaped, fertile on upper half only, up to 16μ in diameter; conidia globose, 1.5μ by 2.5μ in diameter, smooth; sterigmata in two series, primary 8μ to 12μ by 3μ to 5μ; secondary 4μ to 6μ by 2μ to 3μ, compact. Perithecia none. Sclerotia none.

FORM NO. 4

Form 4 (fig. 4) is very closely rated to *Aspergillus ustus*.

Colonies fluffy or more or less cottony at first, turning brown in the center and spreading toward the edge as they grow older; reverse pale yellow at first, becoming deeper yellow to brownish yellow with age. Conidial heads loose, irregular, hemispherical, up to 100μ in diameter. Stalks smooth, colored, rising from aerial hyphae, up to 300μ by 5μ to 8μ. Vesicles developing from slight swellings of the stalk, through flask-shaped to more or less globose, up to 16μ in diameter. Sterigmata in two series, semiradiate. Conidia globose, 3.5μ to 4.5μ, colored, spinulose. Perithecia none. Sclerotia none.

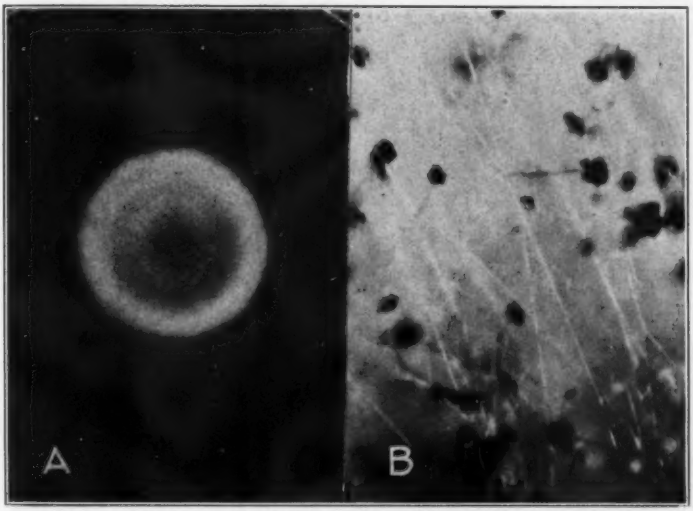

FIGURE 4.—Aspergillus form No. 4. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

FORM NO. 5

Form 5 (fig. 5) belongs to the *Aspergillus niger* group, and because of the large variation within the species, may easily be classed as *A. niger* Van Tieg.

Colonies blackish brown to black; reverse white, wrinkled. Conidial heads globose at first, up to 300μ in diameter, usually splitting with age in two or more ways, forming radiating conidial columns; stalks rising from both submerged and aerial hyphae, up to 2.5 mm. in length and up to 20μ in width, walls yellowish, 3.5μ to 4μ thick, smooth, nonseptate; vesicles globose, up to 60μ in diameter; conidia globose, 3.5μ to 4μ in diameter, colored, spinulose. Sterigmata in one and two series, primary sterigmata up to 60μ in length and up to 4μ in width, secondary sterigmata up to 8μ by 2μ or 3μ. Perithecia none. Sclerotia none.

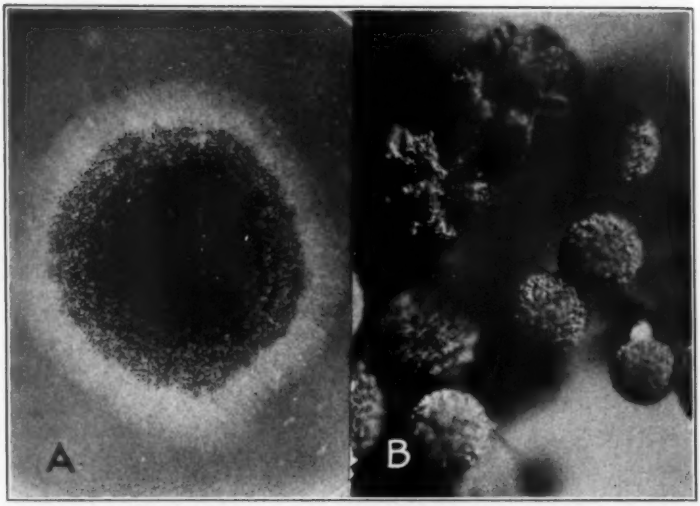

FIGURE 5.—Aspergillus form No. 5. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

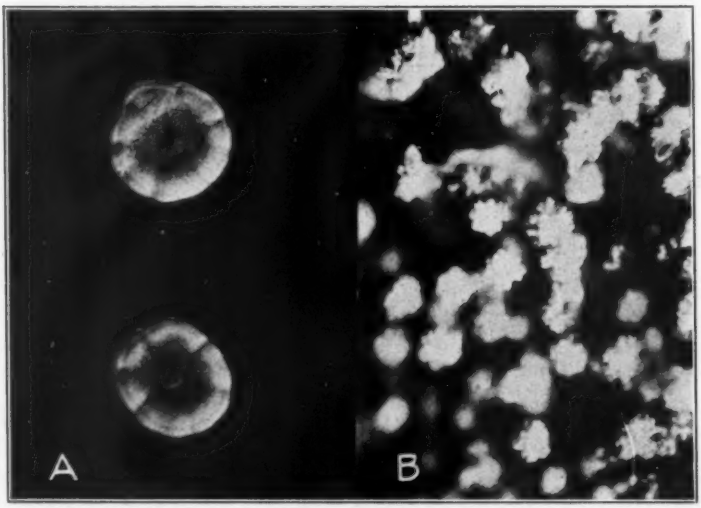

FIGURE 6.—Aspergillus form No. 6. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

FORM NO. 6

Form 6 (fig. 6) is very closely related to *Aspergillus sydowi* (Bainier and Sartory) T. and C., and is perhaps identical with it.[7]

Colonies blue-green, sometimes with age turning a more or less rusty color in the denser portions; surface convoluted; reverse at first orange, becoming deep red with age. Conidial heads loosely globose or radiate, up to 160μ in diameter. Stalks rising from more or less submerged hyphae except in center of colony, where they rise from aerial hyphae, up to 500μ by 8μ, colorless, smooth, septate; vesicles globose to flask-shaped, up to 20μ in diameter; sterigmata radiate in two series, primary 4μ to 8μ by 2μ to 3μ, secondary 4μ by 2μ; sometimes in one series, 6μ to 10μ by 2μ to 4μ. Conidia globose, 2μ to 3μ in diameter, spinulose. Perithecia none. Sclerotia none.

FORM NO. 7

Form 7 (fig. 7) is perhaps identical with *Aspergillus terreus* Thom as described by Thom and Church.

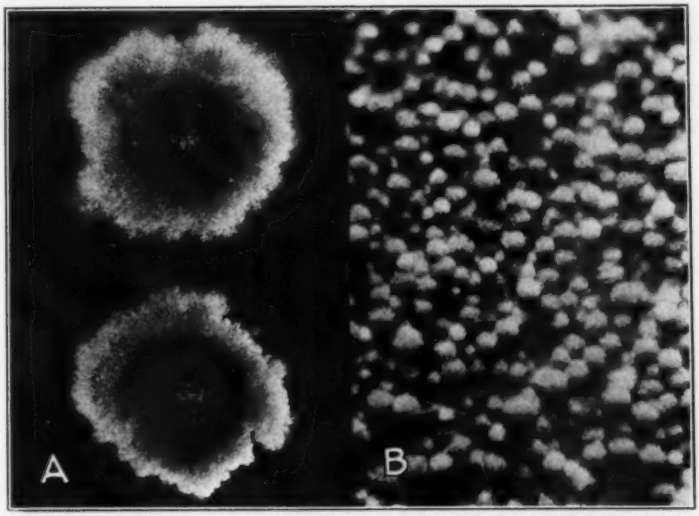

FIGURE 7.—Aspergillus form No. 7. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

Colonies light pink around the edges, deepening toward the center becoming cinnamon-rufous brown (Ridgway,[8] Pl. XIV), in old cultures. Conidial heads columnar, up to 300μ long and up to 85μ wide, densely crowded. Stalks short, up to 130μ long and up to 8μ wide, rising from submerged hyphae, more or less flexuous, smooth, densely crowded. Vesicles more or less flask shaped, up to 18μ in diameter. Sterigmata in one or two series. Conidia globose, 2μ to 3μ, smooth. Perithecia none. Sclerotia none.

FORM NO. 8

Form 8 (fig. 8) belongs to the *Aspergillus fumigatus* group. Identification was not carried farther because of the lack of perithecial production upon which further division might have been made (Thom and Church[9]).

Colonies bluish green at first, turning to dark green with age; reverse yellowish green. Conidial heads columnar, compact, up to 300μ long, mostly about 200μ long, and up to 68μ wide. Stalks rising from submerged hyphae, smooth, slightly colored, nonseptate, up to 500μ long and up to 12μ wide; vesicles flask-

[7] THOM, C., and CHURCH, M. B. Op. cit., p. 147–148.
[8] RIDGWAY, R. Op. cit.
[9] THOM, C., and CHURCH, M. B. Op. cit.

shaped, up to 26μ in diameter, fertile only on upper half; sterigmata in one series, 6μ to 8μ by 2μ to 3.5μ, closely packed; conidia globose, 2μ to 4μ in diameter spinulose. Perithecia none. Sclerotia none.

FORM NO. 9

Form 9 (fig. 9) may be referred very definitely to the *Aspergillus glaucus* group.

Colonies light gray-green near edge, deepening toward the center (glaucous-green, Ridgway, P. XXXIII);[10] reverse cream colored; growth regular. Conidial heads loosely columnar, up to 250μ in length, width varying greatly due to looseness of head, averaging 50μ; conidial chains spreading and more or less tangled. Stalks rising from submerged hyphae, varying from a few to 160μ long by 6μ wide, smooth, septate, sometimes swelling toward the vesicle, and in a few cases, branching near the tip, thus producing two heads. Vesicles ranging in shape from slightly apical swellings of the stalks or flask-shaped to more or less globose;

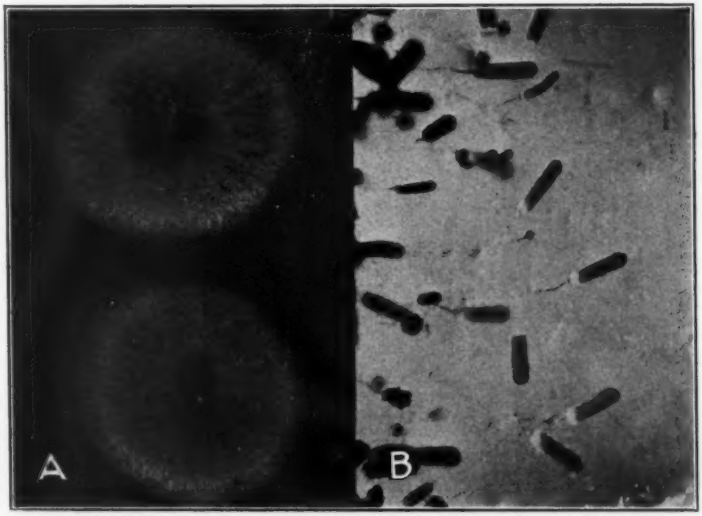

FIGURE 8.—Aspergillus form No. 8. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

up to 16μ in diameter, in many cases proliferating to form short secondary stalks with small heads. Sterigmata one series, short. Conidia globose, up to 4μ in diameter, smooth. Perithecia none. Sclerotia none. Chlamydospores numerous.

FORM NO. 10

Form 10 (fig. 10) belongs to the *Aspergillus nidulans* group, and is perhaps identical with *A. nidulans*.

Colonies green, edges irregular, yellow perithecia soon developing singly, scattered over entire colony; reverse light brown at first, turning reddish brown. Conidial heads columnar, compact, up to 300μ long and up to 60μ wide; stalks smooth, more or less flexuose, branched in some cases, yellowish brown, septate, up to 100μ long and up to 8μ wide; vesicles domelike swellings at end of conidiophores, up to 15μ in diameter; sterigmata in two series, primary up to 8μ by 2μ to 3μ, secondary up to 5μ by 2μ. Conidia globose 3.5μ to 4.5μ in diameter, spinulose. Perithecia up to 650μ in diameter including the Hülle cells, almost black; asci numerous, globose, 8 spored, 10μ to 12μ in diameter; ascospores globose to elliptical 3.5μ to 4.5μ. Sclerotia none.

[10] RIDGWAY, R. Op. cit.

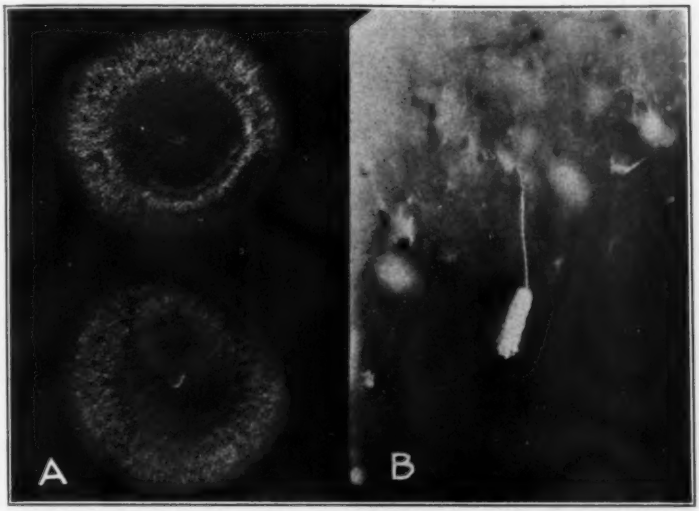

FIGURE 9.—Aspergillus form No. 9. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

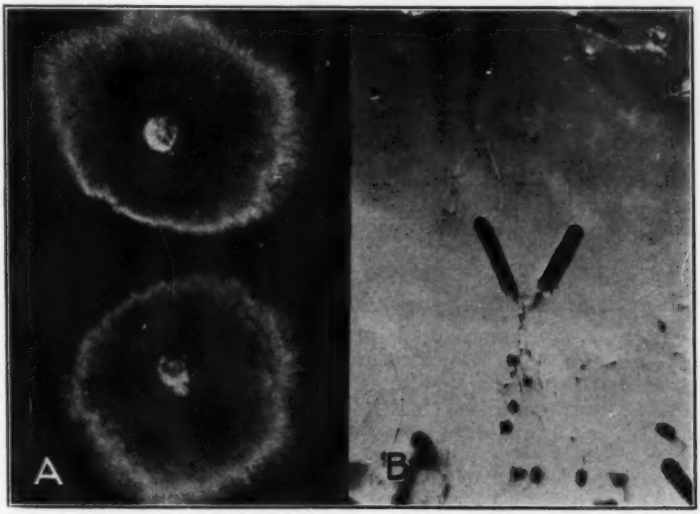

FIGURE 10.—Aspergillus form No. 10. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

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FORM NO. 11

Form 11 (fig. 11) resembles *Aspergillus sydowi* (Bainier and Sartory) T. and C. in colony characteristics but differs somewhat in other respects.

Colonies blue green, tending toward green with age; reverse light brown with brown center, becoming reddish brown with age. Conidial heads varying greatly, loosely hemispherical with radiating chains to very loosely columnar. Resembles *Aspergillus sydowi* as described by Thom and Church [11] in reduced conidial apparatus frequent as small heads, pencillate clusters of branched sterigmata or short conidiophores or single branched sterigmata sessile on trailing hyphae. Stalks smooth, sparsely septate, rising mostly from submerged hyphae, up to 500μ by up to 6μ. Vesicles more or less flask shaped, upper half fertile, up to 15μ in diameter. Sterigmata in two series, primary and secondary nearly equal

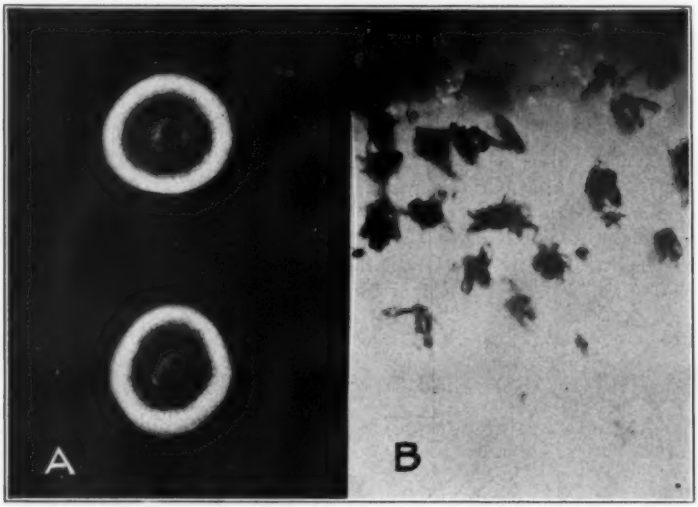

FIGURE 11.—Aspergillus form No. 11. Plate culture on Czapeks' solution agar incubated at 20° C. for eight days (A), and photomicrograph of a portion of a colony showing type of conidial heads (B). × 50

in size, up to 18μ by up to 3μ. Conidia globose, 3μ to 4μ in diameter, spinulose. Perithecia none. Sclerotia none.

INOCULATION EXPERIMENTS

To determine the pathogenicity of the various forms of Aspergilli described above a number of inoculation experiments were made. In these experiments Jonathan apples were used. The apples were packed in Wenatchee and shipped to Pullman, where they were kept in storage until used. All the apples were from the same lot and were as nearly alike in size and degree of maturity as could be secured.

Brooks and Cooley [12] state that the rapidity of rotting depends greatly upon the variety and maturity of the fruit. The writer has also found this to be the case, particularly with the weaker organisms.

Inoculations from single-spore cultures of the forms described above were made according to a method worked out by the author.[13] The

[11] THOM, C., and CHURCH, M. B. Op. cit., p. 148.
[12] BROOKS, C., and COOLEY, J. S. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. Jour. Agr. Research 8: 139–164, illus. 1917.
[13] HUBER, G. A. A SIMPLE METHOD OF INOCULATING THE APPLE. Phytopathology 20: 101–102. 1930.

inoculated fruits were placed under various conditions of storage, and subsequent reisolations were made of the forms causing decay to establish their pathogenicity.

Six apples were inoculated. Three inoculations were made in each with each of the 11 forms of Aspergillus and the fruits were placed in each of the 11 forms of Aspergillus and the fruits were placed in the cold-storage room, where the temperature was maintained at about 0° C. A second lot of apples, inoculated as above, was placed in the common storage room in which the temperature ranged from 10° to 12°; a third lot was placed in the plant pathology storeroom, in which the temperature ranged from 18° to 22° C. The rate of decay caused by the different forms at temperatures of 10° to 12° and 18° to 22° is given in Table 2. The results here presented show that seven of the forms are capable, under favorable storage conditions, of causing decay when inoculated into sound apples. The pathogenicity of some of the forms is very weak, even under the most favorable storage conditions, while others develop rapid decay. That the rate of decay varies greatly in the different forms is seen in Figures 12 and 13.

Under cold-storage conditions (about 0° C.) none of the forms of Aspergillus had caused decay by the end of 12 weeks, when the final examination was made. It is a well-known fact that species of Aspergillus are relatively high-temperature organisms; therefore it was not expected that decay would develop at such low temperatures. As a matter of fact, in the three years during which storage-rot investigations have been in progress at this station, no form of Aspergillus has been cultured from more than a thousand isolations made from rots of cold-storage apples. Apples were inoculated with *Aspergillus niger* (form 5), which is the most rapid rot-producing form of Aspergillus, and incubated at 5° in the ice chest. At this temperature also the organism failed to produce decay at the end of 12 weeks.

TABLE 2.—*Rate of decay caused by forms of Aspergillus in apples in common storage and storeroom temperatures*

Aspergillus form No.	Rate of decay at a temperature of 10° to 12° C.		Rate of decay at a temperature of 18° to 22° C.		Aspergillus form No.	Rate of decay at a temperature of 10° to 12° C.		Rate of decay at a temperature of 18° to 22° C.	
	Average decay per week	Total decay in 12 weeks	Average decay per week	Total decay in 4 weeks		Average decay per week	Total decay in 12 weeks	Average decay per week	Total decay in 4 weeks
1	[1]	[1]	0.9	3.6	7	0.083	1	0.925	3.7
2	[2] 0.075	0.9	.35	1.4	8	[1]	[1]	[1]	[1]
3	[1]	[1]	.35	1.4	9	?	[4]	.4	1.6
4	[1]	[1]	[1]	[1]	10	?	[4]	.45	1.8
5	.258	3.1	2.8	[3]	11	?	[1]	[1]	[1]
6	[1]	[1]	[1]	[1]					

[1] No decay.
[2] The figures represent the surface diameter of the rotted areas in centimeters.
[3] Entire apple decayed.
[4] Decay starting.

DESCRIPTION OF DECAY CAUSED BY FORMS OF ASPERGILLUS

The following descriptions give the character and type of decay produced by the pathogenic forms. These were taken from decayed areas resulting from inoculations made January 18, 1929, and incubated for one month at storeroom temperature ranging from 18° to 22° C.

FORM NO. 1.—The exterior of the decayed portion is a deep brown in color, showing a tendency to form concentric rings which in some cases become quite pronounced. (Fig. 13, A.) The decayed flesh is brown, firm, and moist, although it appears somewhat dry. The decayed area generally has the form of a slightly flattened cone, the diameter at the surface of the fruit exceeding the depth to which it penetrates. (Fig. 14, A.)

FORM NO. 2.—The surface of the decayed area is somewhat sunken, with two distinct zones. On light-colored apples, the inner zone is more or less reddish brown, while the outer zone is usually a light brown. On darker colored apples the outer zone becomes darker, and is a dark red in highly colored apples in contrast to the reddish brown inner zone. (Fig. 13, B.) The decayed flesh is brown, very dry, and corky in appearance. The tissue is tough and has a somewhat bitter taste. The organism advances into the tissue very irregularly; however, the decayed area has a more or less conical appearance. (Fig. 14, B.)

FIGURE 12.—External view of decay in Jonathan apple showing concentric rings caused by Aspergillus form 5

FORM NO. 3.—This form produces a very peculiar type of rot. There is no sign of decay on the surface of the apple until the rot has advanced considerably within the flesh. At first it has the appearance of a water-soaked area, without definite shape. Later as the infected area enlarges it becomes light brown in color, although the edges still retain the water-soaked appearance and very irregular outline. (Fig. 13, C.) The affected tissue is light brown in color, firm, more or less moist, and subglobose in shape. The infection takes place at the bottom of the puncture and spreads more rapidly toward the interior than toward the exterior of the apple. (Fig. 15, A.)

It was difficult to determine the rate at which the decay advanced with this form because of the peculiar type of development. Table 2 shows the measurement of the decay when the final record was taken.

FORM NO. 5.—This form causes a much more rapid decay than any of the others. The exterior of the decayed area shows a distinct

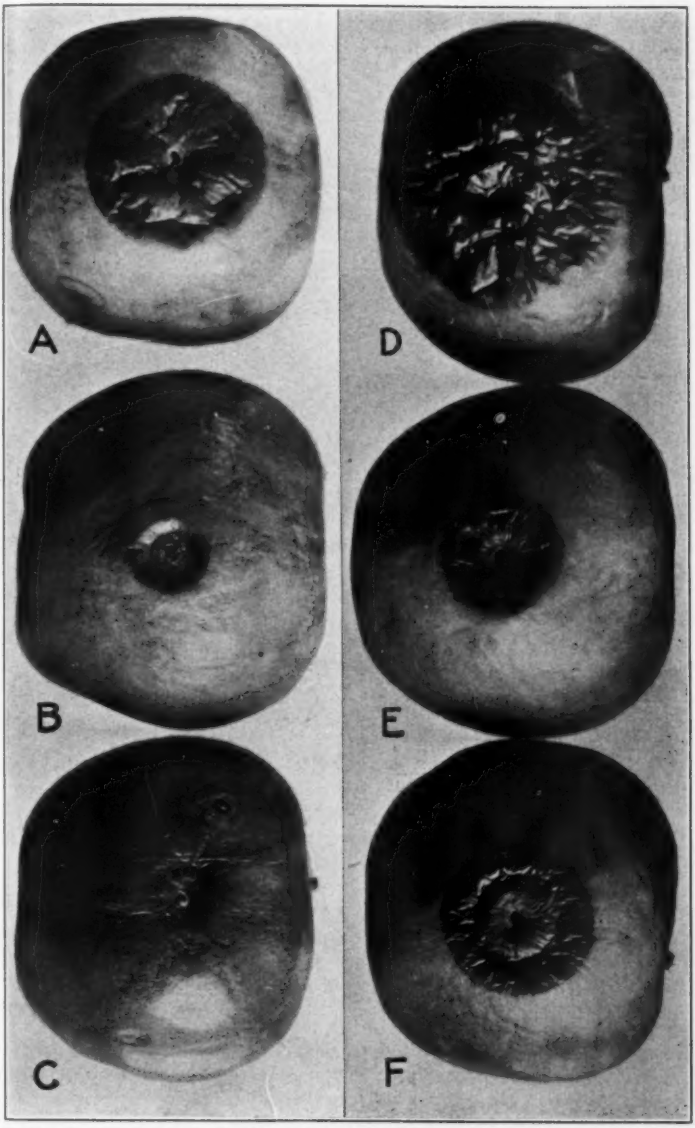

FIGURE 13.—Jonathan apples showing external view of decays caused by forms of Aspergillus. Decay caused by Aspergillus form No. 1, (A); No. 2, (B); No. 3, (C); No. 7, (D); No. 9, (E); No. 10 (F)

zonation in a large majority of cases. (Fig. 12.) As the decay advances, the original color of the apple disappears and the decayed area becomes very light in color with a pinkish cast. The pinkish coloration is much deeper in highly colored apples than in those with little color. The decayed tissue is extremely soft and watery, but there is no sign of the fungus on the unbroken epidermis over the decayed area. The decayed area is conical in shape, the point of the cone soon extending to the core of the apple. (Fig. 15, B.)

FORM NO. 7.—The decay caused by this form is very similar to that caused by form 1. The exterior is brown with a slight tinge of green, but it does not show the concentric rings that generally appear in form 1. (Fig. 13, D.) The decayed flesh is also brown with a light greenish cast. It is conical in shape, extending much deeper

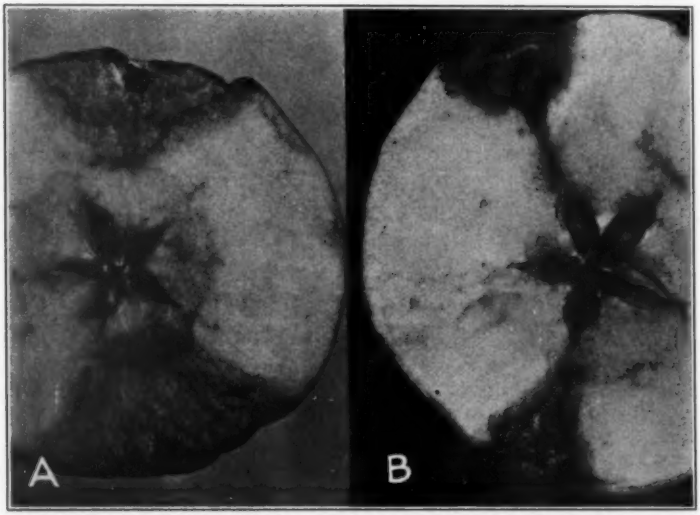

FIGURE 14.—Cross section of Jonathan apple showing type of decay caused by Aspergillus forms No. 1, (A), and No. 2, (B)

into the fruit in proportion to the diameter at the surface than the decay caused by form 1. (Fig. 16, A.)

FORM NO. 9.—The surface of the decay caused by this form is greenish brown. The margin of the decay fuses more or less with the healthy portion, giving no distinct line of separation between the two. (Fig. 13, E.) The decayed flesh is brown, showing a greenish tinge. It is more or less dry and spongy. The decayed area is somewhat conical in shape, tending toward subglobose in many cases. (Fig. 16, B.)

FORM NO. 10.—The exterior of the decayed portion is brown in color, with a very distinct line of demarcation between healthy and decayed areas. (Fig. 13, F.) The decayed flesh is brown, dry, corky, and irregular in shape. (Fig. 17, A.)

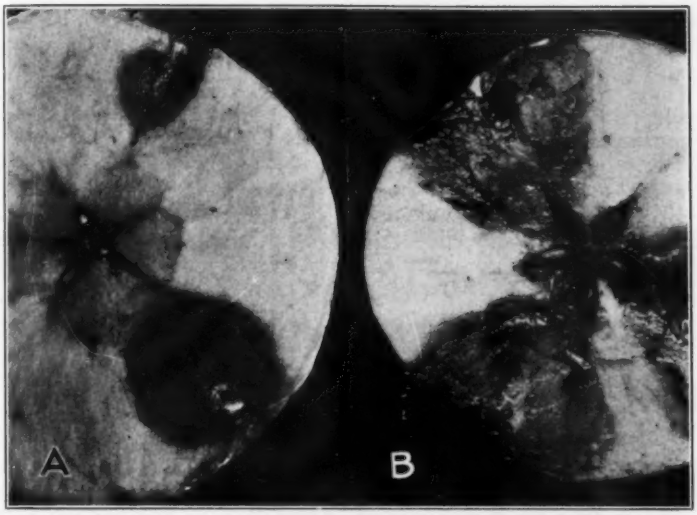

FIGURE 15.—Cross section of Jonathan apple showing type of decay caused by Aspergillus forms No.3 (A) and No. 5 (B)

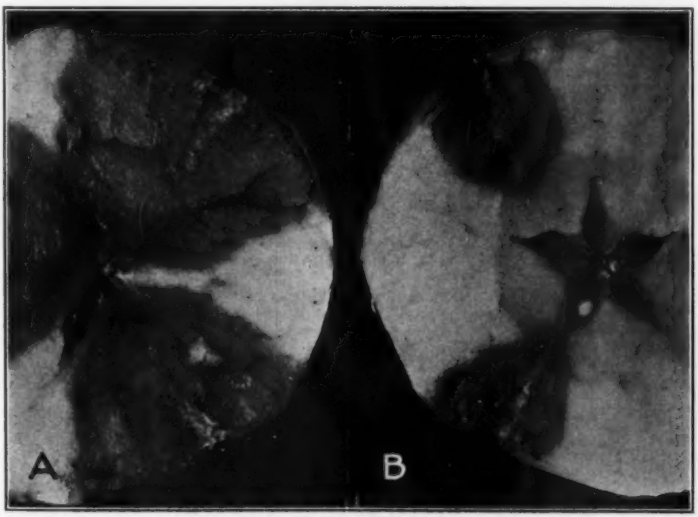

FIGURE 16.—Cross section of Jonathan apple showing type of decay caused by Aspergillus forms No. 7 (A) and No. 9 (B)

TEMPERATURE RELATIONS OF ASPERGILLUS FORMS IN CULTURE

Experiments were made to determine the temperature relations of the different forms of Aspergillus. Czapeks' solution agar was used as the culture medium. Fresh cultures of the forms were made from single-spore stock cultures, that they might be in a thrifty growing condition before the plantings were made.

On each of two plates three plantings of each of the 11 forms were made and the plates were then placed in the cold-storage room (temperature 0° C.). Two more sets of plate cultures were made in the same manner. One set was placed in the common storage room (temperature 10° to 12°) and the other set was placed in the plant-pathology storeroom (temperature 18° to 22°). These conditions of incubation were the same as those under which the inoculation experiments were carried on. The rate of growth of the 11 forms at temperatures of 10° to 12° and 18° to 22° are given in Table 3.

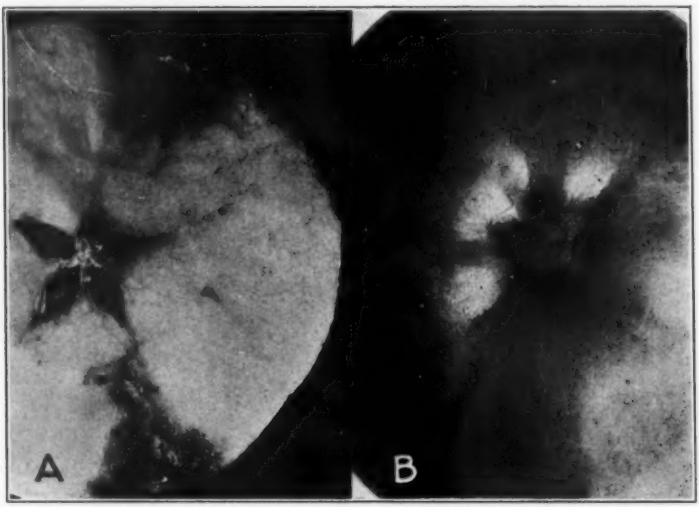

FIGURE 17.—A, Cross section of Jonathan apple showing type of decay caused by Aspergillus form No. 10; B, Jonathan apple taken from cold storage showing mycelial growth in calyx end

TABLE 3.—*Comparative rate of growth of Aspergillus forms on Czapeks' solution agar in common storage and at storeroom temperatures*

Aspergillus form No.	Growth in common storage at a temperature of 10° to 12° C.		Growth at storeroom temperatures of 18° to 22° C.		Aspergillus form No.	Growth in common storage at a temperature of 10° to 12° C.		Growth at storeroom temperatures of 18° to 22° C.	
	Average growth per day	Total growth, 24 days	Average growth per day	Total growth, 8 days		Average growth per day	Total growth, 24 days	Average growth per day	Total growth, 8 days
1	[1] 0.104	2.5	0.425	3.4	7	0.083	2.0	0.375	3.0
2	.125	3.0	.4	3.2	8	.062	1.5	.463	3.7
3	.033	.8	.275	2.2	9	.141	3.4	.463	3.7
4	.15	3.6	.4	3.2	10	.033	.8	.413	3.3
5	.166	4.0	.563	4.5	11	.158	1.4	.213	1.7
6	.062	1.5	.188	1.5					

[1] The figures represent the surface diameter of the rotted areas in centimeters.

At the end of four weeks no growth had appeared on the plantings kept at 0° C. The cultures were then taken to the laboratory and held for several weeks. Growth was somewhat delayed in comparison with that of cultures incubated at room temperature immediately after planting.

All the forms made some growth at temperatures of 10° to 12° C., although the growth was much less than that at 18° to 22°. Some forms grew much more slowly at common storage temperatures than other forms. This is particularly true of forms 3, 8, and 10. Forms 2 and 4 developed much more rapidly at the lower temperatures than did the other forms.

SUMMARY

Apples washed in sterile water with sterile, stiff stencil brushes for five minutes, and the wash water plated out in potato-dextrose agar, yielded from less than 1,000 up to more than 271,000 fungous colonies per apple.

Eleven forms of Aspergillus which have been isolated from the surface of normal apples are described and placed in form groups.

Seven of the eleven forms caused decay when inoculated into normal apples. None of the forms caused decay under cold-storage conditions (0°C.). (Forms 2, 5, and 7 caused decay under common storageroom conditions at temperatures of 10° to 12°.) Forms 1, 2, 3, 5, 7, 9, and 10 caused decay under storage conditions at temperatures of 18° to 22° C.

The types of decay caused by the different forms of Aspergillus vary greatly. Some forms cause firm and more or less moist rots, others dry and somewhat corky or leathery rots, while *Aspergillus niger* (form 5) causes a very soft and watery rot.

The forms of Aspergillus in culture developed more rapidly in storage at temperatures of 18° to 22° C. than at temperatures of 10° to 12°. No growth appeared in any of the forms in cold storage (0°).

CHANGES IN THE PHOSPHORUS CONTENT OF GROWING MUNG BEANS [1]

By JAMES E. WEBSTER, *Associate Professor*, and CLAUDE DALBOM, *Instructor*, *Department of Agricultural Chemistry Research, Oklahoma Agricultural Experiment Station*

INTRODUCTION

As one phase of an investigation of the rôle of phosphorus in plants, a study was made of the variations in the content of certain forms of phosphorus which occur in the chief aerial organs of the mung bean (*Phaseolus aureus*) at different stages in its development. The study included plants grown in the field and in test plots treated with superphosphate at the rate of 600 pounds per acre. It is believed that the figures here presented will be of interest to workers in the field of plant chemistry, and that the data may be used to check the results secured from a study of this and other forage crops when grown under controlled conditions of nutrient supply. The phosphorus content is of interest also in relation to the time of harvest, since it is thought that there is a phosphorus deficiency in feeds from certain sections and consequently it would be desirable to harvest the crop when the greatest phosphorus content could be secured.

In a previous paper (*8*)[2] from this station the phosphorus fractionation of the mung bean was given together with that of other seeds. Inorganic phosphorus was shown to be a relatively unimportant part of the total phosphorus in the bean, but in the present work with the green plant the inorganic changes were found to be most significant. Because of the color obtained in the solutions, it was impossible to use the iron titrimetric method for phytin; therefore, this determination has been omitted, and the chief comparison will be made between total, inorganic, and organic forms of phosphorus. Since the amounts of lipoid phosphorus are small, detailed figures are not given but in their stead are shown the average of the percentages found on the various dates.

WORK OF OTHER INVESTIGATORS

Zlataroff (*9*) followed the changes in various forms of phosphorus in seedlings of the chickpea (*Cicer arietinum*) grown in water for 25 days. During this time he found that the total and inorganic phosphorus increased, while lecithin, protein, and the soluble organic forms decreased. Not all of these changes continue as plants grow, however, as the present work will show.

André (*1*) gives figures for phosphorus distribution at four stages in the growth of an annual plant, the poppy. From his analyses he finds that the maximum percentage of phosphorus occurs in roots, stems, and leaves at blossoming time and thereafter decreases. In the fruit phosphorus increases until the plant is nearly mature, after which the change is slight.

[1] Received for publication June 24, 1930; issued December, 1930. Published with the permission of the director of the experiment station.

[2] Reference is made by number (italic) to Literature Cited, p. 824.

Hartwell and Hammett (*7*) studied the effect of phosphorus manuring on turnips and found that while increasing the applications of phosphorus increased the total phosphorus twofold, it increased the inorganic phosphorus sixfold.

Greaves and Hirst (*4*) studied the effect of irrigation on the relation between total and inorganic phosphorus in wheat, oats, barley, and corn. In all cases they found that the increase of total phosphorus, when the amount of water was increased, was chiefly organic in form. They also found that the percentage of inorganic phosphorus was low in these plants and did not vary greatly in different sections.

MATERIALS AND METHODS

The mung bean plants used in this work were grown during the summers of 1928 and 1929. The plants used for the biweekly samplings in 1928 were selected from an unfertilized field of about 2 acres, while those used in the fertilizer studies of 1929 were selected from one-sixtieth-acre plots. All plants were sampled at approximately 1.30 p. m., and samples large enough to give from 50 to 200 gm. of dried material were used except in the case of the first few samplings, when a representative sample could be secured from less material. In no case were less than 10 plants collected for a sample, although in some of the later samplings when the plants were very large not all of the entire 10 plants were used for a sample.

As soon as the samples were cut they were brought in from the field, and the leaves, main stalk, and branches were separated and placed in trays in a hot-air drier. The drier was maintained at a temperature sufficient to dry the samples in eight hours and yet not injure the tissue by charring. After eight hours in the drier the samples were placed in an oven maintained at 110° C., and brought to uniform dryness. They were then ground to pass the fine-mesh screen of the Wiley mill.

The total phosphorus determinations were made according to the directions of the Association of Official Agricultural Chemists (*2, p. 2, par. 6; p. 3, par. 10A*).

Inorganic phosphorus was determined according to the directions of Collison (*3*), and while this method may have errors (*4*) the results are comparative and probably closely approach the real values.

For the estimation of lipoid phosphorus the microcolorimetric method of Guerrant (*5*) was used. The following modifications, however, were made to secure better results (*6*). Two-gram samples of dried material were extracted with 80 c. c. of the alcohol-ether mixture by shaking for 15 minutes and digesting at 30° C. for two hours, filtering, and making up to 100 c. c. Ten cubic centimeters were used for each determination.

The figures for organic phosphorus represent the difference between the total and the inorganic phosphorus.

Ether extract was secured by extracting dried samples with anhydrous ether in a drip extractor until constant in weight. Even with this procedure it was impossible to secure consistent results with the leaves.

Moisture and ash were determined in the conventional manner.

DATA AND DISCUSSION

Table 1 shows the seasonal distribution of phosphorus in the forms for which analyses were made and the ratio of organic to total phosphorus. This ratio is of value in that it shows how the phosphorus is being utilized and gives some indication as to the seat of active phosphorus changes.

One of the most interesting facts shown in the table is that, with the exception of the third and fourth samplings, when growth was most active, the amounts of inorganic phosphorus are relatively constant, especially so when the marked decreases in other forms of phosphorus are noted.

TABLE 1.—*Phosphorus distribution in mung beans grown in the field, 1928.*

[Percentages based on dry matter]

LEAVES

Date	Moisture	Ash	Ether extract	Phosphorus				Ratio of inorganic to total phosphorus
				Total	Inorganic	Organic	Lipoid	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
June 15	77.74	12.46	1.07	0.327	0.039	0.288		0.12
June 26	81.00	11.24	1.98	.492	.040	.452		.08
July 5	78.33	10.77	.86	.474	.066	.388	0.011	.14
July 17	77.33	10.60	1.08	.308	.057	.251		.18
July 30	77.67	10.05	.74	.261	.048	.213		.18
Aug. 13 [a]	74.30	10.45	.70	.202	.043	.159		.21
Aug. 28	74.25	10.07	1.78	.204	.038	.166		.19
Sept. 11 [b]	71.67	9.32	2.12	.122	.030	.092	.01	.25
Sept. 24 [c]	69.60	9.16	1.62	.120	.034	.086		.28
Oct. 17								

BRANCHES

June 26	90.0	13.91	1.79	0.315	0.054	0.261		0.17
July 5	88.0	10.48	1.70	.288	.061	.227	0.011	.21
July 17	83.7	8.89	1.47	.256	.090	.166		.35
July 30	84.3	7.92	1.28	.197	.069	.128		.35
Aug. 13	80.5	5.53	1.98	.146	.050	.096		.34
Aug. 28	83.4	5.33	1.16	.163	.067	.096		.41
Sept. 11	72.3	4.15	1.10	.085	.050	.035	.009	.59
Sept. 24	69.1	3.85	.93	.099	.053	.046		.54
Oct. 17	70.6	3.76	1.19	.094	.041	.053		.44

STEMS

June 15	85.0	14.95	2.58	0.404	0.056	0.348		0.14
June 26	90.0	12.08	2.20	.431	.068	.363		.16
July 5	88.3	7.21	1.85	.373	.097	.276	0.01	.26
July 17	84.3	5.14	1.19	.210	.093	.117		.44
July 30	82.3	5.09	.96	.141	.055	.086		.39
Aug. 13	80.25	3.80	.94	.128	.055	.073		.47
Aug. 28	72.0	3.55	.95	.102	.062	.040		.61
Sept. 11	70.14	3.02	.72	.069	.061	.008	.009	.88
Sept. 24	70.61	3.27	.61	.073	.053	.020		.73
Oct. 17	69.70	3.25	.71	.074	.041	.033		.55

SEEDS AND PODS

Aug 13	83.0	5.88	1.66	0.497	0.070	0.427		0.14
Aug. 28	64.56	5.01	.81	.391	.050	.341		.13
Sept. 11	60.0	4.98	.46	.415	.058	.357	.013	.14
Sept. 24	24.46	4.53	.83	.365	.058	.307		.16
Oct. 17	29.05	4.65	.67	.379	.046	.333		.12

[a] Pods and beans well formed but still green.
[b] Leaves breaking down.
[c] Many leaves fallen; others yellow.

The ash determinations are included in the tables to show the relation of total phosphorus to the other mineral elements. In Table 1 it will be noted that the ash content of the leaves falls only about 20 per cent of its value from the June 26 sampling until the last sampling, while the decrease in total phosphorus for the same period was 75 per cent. The reason for this is not apparent, although it was noted when digesting the samples for total phosphorus that there was much more silica in the leaves than in the other organs. This fact may in part be accounted for by the smaller ash content of the other organs, but even this would not seem to account for the entire difference. It was expected when this study was started that a much larger percentage of lipoid phosphorus would be found in the leaves than in other organs, but such is not the case. Since percentages of this form are small and only slight changes occur during the life cycle of the plant, individual figures are not given; instead, the average figure for the early stages and the average for the late stages of growth are given except in the case of the seeds and pods, where a single average is shown.

Another notable fact which is in accord with the idea that the leaf is the seat of synthetic processes in plant metabolism is shown by the relatively large fraction of the leaf phosphorus that is present in some organic form. Since the lipoid form does not account for a large percentage of the whole, it is unfortunate that we do not, in these analyses, have a direct measure of the phosphoproteins of the leaf. Certainly it is true that as long as the leaves are green, organic phosphorus forms constitute the major fraction. The discrepancies shown in the August 13 sampling can probably be explained by assuming a sampling error, since the inequalities run through each analysis at that time.

As would be expected, the values for phosphorus in the stems and branches are similar (Table 1), and show a considerably greater proportion of the total phosphorus in the inorganic form than is found in the leaves. This relationship of the inorganic phosphorus seems to be rather directly related to the type of tissue. The greater the growth activity, the less inorganic phosphorus there is present. Certainly there is no evidence of a transfer of phosphorus from the leaves or branches to the stems. This would seem to indicate a progressive movement to the leaves, since the decrease is least there if the last two samplings made when the leaves had begun to disintegrate are omitted. It is particularly notable that in the case of stalks and branches the decrease in phosphorus is in much the same ratio as the changes in ash content. This fact may be taken as further evidence in favor of the view that the localization of phosphorus is the result of the normal intake of mineral elements and is not due to particular metabolic processes.

Only slight changes occur in the various forms of phosphorus as the plant ages. (Table 1.) This may be due in part, at least, to the great rapidity with which the pods ripen. Since the phosphorus content of the plant varies so little at this stage, a difference of two or three weeks in time of harvesting the beans should not appreciably affect their value as feed.

Table 2 shows a comparison between the phosphorus distribution of plants grown in an unfertilized field (check) and those grown on plots fertilized at the rate of 600 pounds of superphosphate per acre.

In addition to the results reported in this table analyses were made of plants variously fertilized with nitrogen and potassium fertilizers. The results of these analyses are not given since they would not significantly alter the percentages of phosphorus here shown.

Perhaps the most important fact brought out in Table 2 is that the use of this high phosphorus fertilizer did not increase the amount of total phosphorus in the plants so fertilized appreciably above that in the plants grown in the checks, except in the case of the leaves and of the pods and seeds in the late sampling. The season was very dry, however, and this may in part account for the fact that the differences were so small. There was little difference in the appearance of the plots or in the amount of growth. It was supposed that the addition of the fertilizer would increase the inorganic phosphorus in the plant, but, as the figures show, the increase is only in the late sampling and the amount of it is small.

TABLE 2.—*Phosphorus distribution in mung beans grown on checks and plots fertilized with 600 pounds superphosphate*

[Percentages based on dry matter]

EARLY SAMPLING; PLANTS 8 TO 14 INCHES HIGH

Plot	Part of plant	Moisture	Ash	Ether extract	Phosphorus			Ratio of inorganic to total phosphorus
					Total	Inorganic	Organic	
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
Superphosphate.	Leaves	80. 10	12. 86	2. 29	0. 390	0. 051	0. 339	0. 13
	Branches	86. 54	14. 36	1. 38	. 275	. 081	. 194	. 29
	Steams	85. 25	13. 75	1. 05	. 226	. 062	. 164	. 27
Check	Leaves	77. 77	13. 03	2. 17	. 380	. 051	. 329	. 13
	Branches	85. 96	14. 07	1. 74	. 293	. 094	. 199	. 32
	Stems	86. 04	11. 37	1. 24	. 237	. 076	. 161	. 32

LATE SAMPLING; SEED PODS NEARLY MATURE

Superphosphate.	Leaves	74. 66	11. 07	2. 08	0. 247	0. 036	0. 211	0. 15
	Branches	78. 66	6. 53	. 99	. 150	. 045	. 105	. 30
	Stems	76. 32	4. 95	. 67	. 142	. 046	. 096	. 32
	Pods and seeds	81. 60	5. 57	. 92	. 335	. 061	. 274	. 18
Check	Leaves	75. 67	11. 64	1. 79	. 216	. 031	. 185	. 14
	Branches	79. 34	7. 09	. 78	. 150	. 038	. 112	. 25
	Stems	76. 50	5. 44	. 68	. 148	. 040	. 108	. 27
	Pods and seeds	80. 33	5. 28	1. 73	. 300			

SUMMARY

A study was made of the phosphorus content of the various aerial organs of the mung bean as the plant grew to maturity. One set of analyses involved the use of plants grown in an unfertilized field and the other that of plants grown on plots fertilized at the rate of 600 pounds of superphosphate per acre. The determinations for certain phosphorus fractions, ether extract, and ash content are shown.

A most interesting finding is the relatively small change that takes place in the amount of inorganic phosphorus present in any one organ. In fact the amount of inorganic phosphorus in any organ is relatively constant except when the plant is small, and apparently

phosphorus as taken into the plant is metabolized to organic forms for storage.

In the green plant lipoid phosphorus constitutes only a small fraction of the whole and varies little at different stages in the growth of the plant. Apparently it is not a storage form of phosphorus in green plants.

At about the usual harvest time there is only a slight change in the total phosphorus content, so that differences of two or three weeks would make only negligible changes in the phosphorus feeding value.

On the basis of quantities present there is no evidence that phosphorus is transferred from the leaves and branches to the stems, and it is assumed that the localization of phosphorus follows the normal ash intake and is not the result of metabolic activities.

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